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Vol. XLIX *August, 1925* *No. 2*

BIOLOGICAL BULLETIN

MORPHOLOGY AND MITOSIS IN *TRICHOMONAS TERMOPSIDIS*, AN INTESTINAL FLAGELLATE OF THE TERMITE, *TERMOPSIS*.[1]

JUSTIN M. ANDREWS,

INTRODUCTION.

In recently published papers by Cleveland ('24 and '25) establishing the symbiotic relationship of termites and their intestinal protozoa, and the effects of starvation and oxygenation upon this association, he mentions that he has observed in the intestine of termites of the genus *Termopsis*, a flagellate possessing "four anterior flagella, axostyle, and undulating membrane" which he calls "*Trichomonas termopsidis*." The object of this paper is to describe the morphology and mitosis of this species as it occurs in the large Pacific Coast termites *Termopsis nevadensis* Hagen and *T. angusticollis* Hagen.

MATERIAL.

Sixteen colonies of *Termopsis* furnished the material for this study. Six of the colonies came from Oregon, and ten were from California. Winged forms appeared in two of the Oregon colonies, and were identified as *T. nevadensis* Hagen. Winged forms appeared in five of the California colonies which were identified as *T. angusticollis* Hagen. The insect material was identified by Dr. T. E. Snyder, Specialist in Forest Insects, Bureau of Entomology, U. S. Department of Agriculture, Washington, D. C.

[1] From the department of Medical Zoölogy, School of Hygiene and Public Health, Johns Hopkins University. A portion of this work was done at the Biological Laboratory, Cold Spring Harbor, Long Island, New York.

Acknowledgments.

Many thanks are due Dr. R. W. Hegner for his valuable suggestions and criticisms. To Dr. L. R. Cleveland I am especially indebted for furnishing the material used, for suggestions as to methods, and for the oversight which he so generously gave.

Methods.

In order to obtain preparations which contained more than the usual number of the flagellates per unit area, the termites were starved for eight days, at which time the two larger protozoa, *Trichonympha campanula* and *Leidyopsis sphaerica*, which had been previously present in large numbers, had entirely disappeared (Cleveland '25). Then the termites were fed a pure cellulose diet (Whatman Filter Paper No. 43) which resulted in a much greater clarity of the intestinal fluid surrounding the flagellates and relieved the animals of many food inclusions (wood particles) that had previously obscured their internal morphology.

Smears were made from the intestinal contents of termites treated as above. They were fixed in various fluids,—Schaudinn's strong Flemming's, Gilson's, Bouin's, Zenker's, Carnoy's, osmic acid vapor, and chromic acid. These were stained, for the most part, with Heidenhain's iron alum hæmotoxylin; a few were stained with Mallory's Tri-Stain. Others were dry fixed and stained with Wright's stain. Sections of the intestines of termites—both of individuals that had been partially defaunated as above, and of untreated hosts, were fixed in Schaudinn's fluid, Flemming's fluid, chromic acid, and osmic acid vapor. They were stained with iron hæmotoxylin, and with Mallory's Tri-stain. The live animals were also studied under vaselined coverslips.

Morphology.

Shape and Size of Body.

Body shape in *Trichomonas termopsidis* is exceedingly variable, due to the absence of a restraining pellicle, and to the extreme lability of the cytoplasm. From a pyriform shape, it varies through a regular and elongated oval, to a spheroidal contour. The greatest width is usually near the middle of the animal, and varies, in proportion to the length, from 1 to 4.5, to 1 to 1.

The size is also very variable, owing perhaps, to division processes, the figures of which are exceedingly numerous. The majority, though by no means all, of the dividing forms are larger

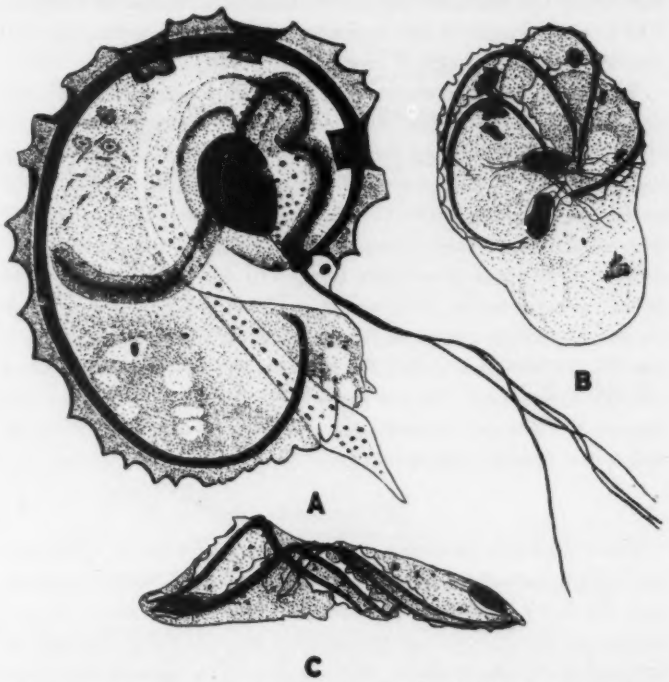


FIG. 1. All figures of *Trichomonas termopsidis* Cleveland, from material stained with iron alum hæmotoxylin, drawn with the aid of camera lucida. Flagella are shown as they appeared in the preparation.

A. Typical trophozoite in resting stage—fixed in 1 per cent. chromic acid. × 2500.

B. Multiple fission, of the type with the nuclei in a central position—fixed in Carnoy's fluid. × 500.

C. Multiple fission, of the type with nuclei at polar extremities—fixed in Carnoy's fluid. × 500.

than the resting individuals. Of one hundred measured as resting trophozoites, the length averaged 29.17 micra, and the width 14.49 micra. The extremes of length were 55.0 micra, and 11.0 micra, the majority of the individuals falling between 25 and 35 micra. The extremes of width were 28.8 micra, and 6.78 micra,

the majority falling between 12 and 20 micra. As these measurements were made from fixed, stained, individuals from partially defaunated hosts, the length measurement was checked by measuring one hundred live individuals from untreated termites. The average length in this series was 30.97 micra, indicating that the treatment has little, if any, effect on size.

The above measurements, and those mentioned hereafter in the description of organelles, were all made from termites of three colonies which furnished most of the material used in the cytological study. When other colonies (thirteen more) were investigated in order to observe the distribution of *Trichomonas termopsidis*, forms were encountered in twelve of the colonies that differed from those previously studied in size, which varied from 50 micra to 150 micra, most of the giant individuals lying between 70 micra and 90 micra in length. In all other morphological details, the two sizes of this *Trichomonas* are identical as far as we are able to observe. So, for the present, we are calling this a size race of *Trichomonas termopsidis*. It may be that further study will reveal specific differences rather than racial differences.

Cytoplasm.

The cytoplasm, as stated above, is extremely labile; indeed it probably approaches fluidity. The shape is constantly changing, and the body assuming new proportions. Occasionly, in live specimens, one can see the animal slide some of the cytoplasm off the end of the axostyle in drops. The cytoplasm is kept in a state of agitation by the activity of the axostyle, which appears to function as an internal paddle, continually churning up the contents of the body.

Cytostome.

The cytostome (*Cytos.*, Fig. 2.) does not appear to be the comma-shaped vent usually pictured for the trichomonads, but extends as a spiral groove starting above the middle on the right-hand side of the animal, becoming more sharply indented as it proceeds ventrally, ending under the nucleus. Although an actual opening cannot be seen, the food particles, and fluid filled vacuoles are more numerous dorsal to the nucleus indicating the position of the opening.

Nucleus.

The nucleus is an ellipsoidal mass enveloped by a membrane which is sometimes closely applied to the darkly staining nucleoplasm, but is more frequently found surrounding it a short

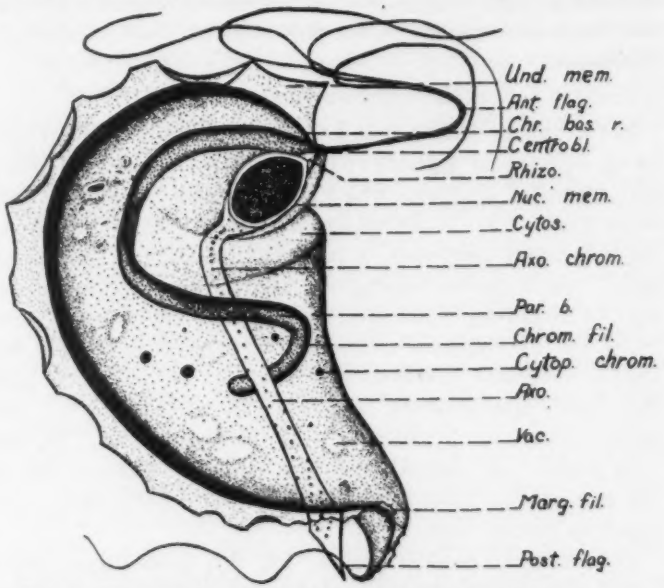


FIG. 2. Diagrammatic representation of *Trichomonas termopsidis*.

Abbreviations: *Und. mem.*, undulating membrane; *Ant. flag.*, anterior flagella; *Chr. bas. r.*, chromatic basal rod; *Centrob.*, centroblepharoplast; *Rhizo.*, rhizoplast; *Nuc. mem.*, nuclear membrane; *Cytos.*, cytostome; *Axo. chrom.*, axostylar chromidia; *Par. b.*, parabasal body; *Chrom. fil.*, chromatic filament; *Cytop. chrom.*, cytoplasmic chromidia; *Axo.*, axostyle; *Vac.*, vacuole; *Marg. fil.*, marginal filament; *Post. flag.*, posterior flagellum. × 3000.

distance away, leaving a clear, unstaining zone between the membrane and the nucleus (Fig. 4, *B* and *C*). Sometimes this zone is not regularly parallel to the contour of the nucleus, but in all such cases observed, the irregularity seems to be due to depressions or elevations in the outline of the nuclear mass, rather than to a collapse of the membrane (Fig. 4, *A* and *D*). The length of the resting nucleus is ordinarily about twice its width. It is always situated anterior to the middle of the body, except in dividing forms, where proportions are upset both in the body of

the individual and in the nucleus. Its length varies from 5 to 10 micra. It stains a flat black unless the preparation is strongly decolorized, in which case it shows a granular composition, within which appears a karyosome-like unit contained in a clear unstaining vesicle (Fig. 4, *A*). In the resting stage, the nuclear membrane is connected to the centroblepharoplast by a single rhizoplast.

Neuromotor Apparatus.

In their paper dealing with *Trichomitus termitidis*, Kofoid and Swezy ('19) list the organelles composing this "complex, structurally integrated apparatus which links together the nucleus and motor organs" as: a centroblepharoplast, the anterior flagella, the undulating membrane, a posterior flagellum, a "parabasal body," and a nuclear rhizoplast. To that category, we would add, in describing *Trichomonas termopsidis*, an axostyle, and would call the organelle referred to as the parabasal body by Kofoid and Swezy, the chromatic basal rod, in view of the fact that a body first called the parabasal body by Janicki ('11), and later corroborated by Alexieff ('13), Kuczynski ('14), Janicki ('15), Cutler ('19), and Wenrich ('21), is plainly demonstrable in our preparations fixed in strong Flemming's fluid, osmic acid vapor, or chromic acid.

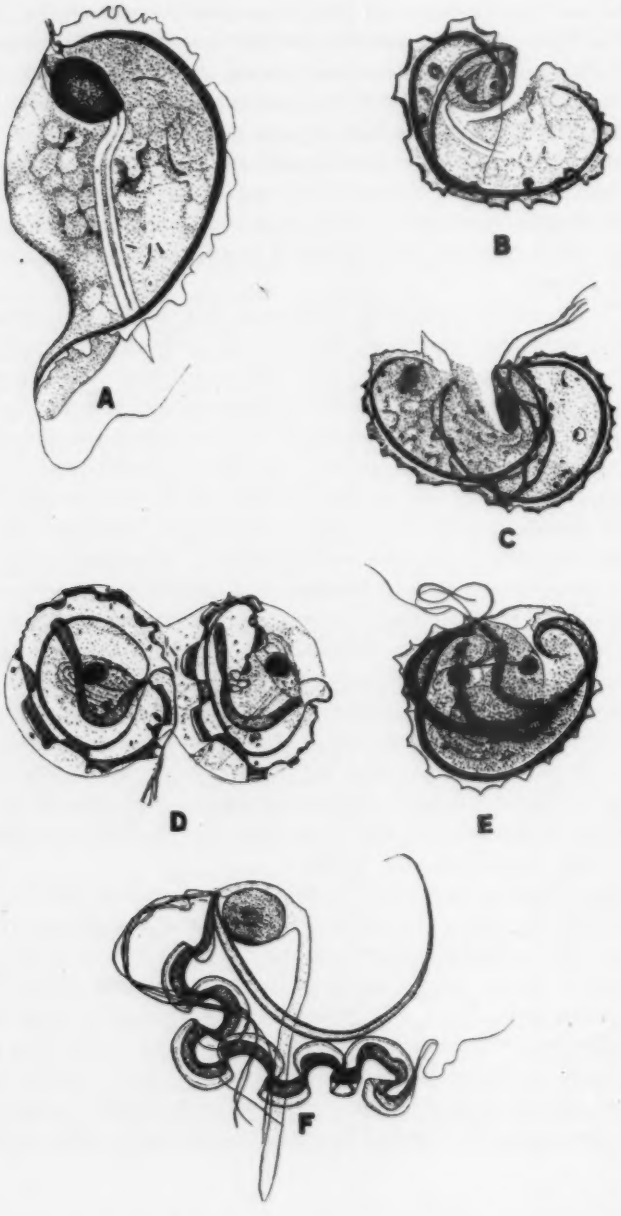
The *centroblepharoplast* (*Centro.*, Fig. 2.), apparently a single morphological unit while the trophozoite is in its resting stage, appears as a small, round, blackly-staining dot about a micron in diameter. It is closely applied to or embedded within the anterior end of the chromatic basal rod, so that except during division processes, its position is marked only by a bulbous enlargement of the extremity of the chromatic basal rod. The centroblepharoplast is always connected to the nucleus by one or more nuclear rhizoplasts. From the centroblepharoplast spring the four anterior flagella, the undulating membrane, the chromatic basal rod, the axostyle, and the parabasal body.

The four *anterior flagella* (*Ant. flag.*, Fig. 2) are about equal in length, arise from the centroblepharoplast, and pass discretely out of the cytoplasm. At the point of emergence, the cytoplasm is usually raised to a small hillock. As the flagella leave the body, they are frequently so intertwisted and woven together, that it

looks as if a single large flagellum proceeded from the body, and, at a short distance, became divided into four. This illusion seems particularly real as one observes the live specimens, but upon careful examination of the stained preparation, it is obvious that the flagella are not only separate outside the cytoplasm, but within it also. The anterior flagella are about three-quarters the length of the body. Specimens stained by Wright's method show the flagella very clearly, and it was not until this technique was employed that the number of flagella was unquestionably established.

The *undulating membrane* (*Und. mem.*, Fig. 2) is a thin, rippling film, apparently composed of a fold of the pellicular covering, attached, at its outer edge, to a posteriorly directed flagellum, which, like the anterior flagella, originates in the centroblepharoplast. The flagellum separates from the membrane at the posterior end of the body, as an inconspicuous trailing flagellum, which is apparently not as well developed as the anterior flagella. The membrane is hyaline, and persists after treatment which dissolves away the cytoplasm of the animal. It is best developed at the anterior end, and becomes less developed and effective towards its posterior end. In preparations stained by Wright's method, the undulating membrane is differentiated into three distinct zones—a distal, narrow, pink-staining line, which is probably the flagellum, then a pellucid, unstained zone approximately half the remaining width of the membrane, and lastly a heavily staining (pink) zone which extends to the attachment (Fig. 3, Fig. 6). A similar differentiation was observed in several specimens stained with iron alum hæmotoxylin that were more critically decolorized (Fig. 3, *D*).

The *chromatic basal rod* (*Chr. bas. r.*, Fig. 2) extends the length of the body, directly beneath the undulating membrane, in a curve that resembles either a capital "C" or "S." It takes its origin from the centroblepharoplast, and like that organelle, stains intensely black. It retains its stain, ordinarily, after the nucleus has become quite decolorized. Whether it functions as a source of energy for the motor activities of the undulating membrane (Kofoid and Swezy, '15) or serves as a skeletal support for that organelle, the fact is that the membrane seems to be


A
B
C
D
E
F

attached directly to the chromatic basal rod. This observation is strikingly demonstrated in those cases where the cytoplasm of the animal has been destroyed, and the entire neuromotor system with, or without the nucleus, remains intact. In the majority of such cases, though not in every one, the undulating membrane remains adjacent to the chromatic basal rod—suggesting that there may be some connection between them which is strong enough to withstand the treatment that destroys the cytoplasm, but which cannot, in a few cases, endure the chance violences of the smearing operation.

The *axostyle* (*Axo.*, Fig. 2) in *Trichomonas termopsidis* is one of its most conspicuous features when the animal is studied in the living state. As the flagellate moves about, turning and twisting, one of the first things to be noted is the projecting trunk of the axostyle. Occasionly it sticks out of the body for a distance as long as the length of the body, but more frequently it barely protrudes through the cytoplasm, the length of the extruded portion being varied both by changing the actual linear expanse of the organelle—which is accomplished by bending it and curving it within the body—but more often by changing the shape of the body, so that more of the axostyle is included at one time than at another.

When the stained preparation is observed, the axostyle is not such a prominent feature. It does not stain well at all. Frequently it does not project beyond the body line, and in such cases it is easily overlooked. Certain fixing fluids show the

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FIG. 3. All figures of *Trichomonas termopsidis* sp. nov., from material stained with iron alum haemotoxylin, drawn with the aid of camera lucida. Flagella are shown as they appeared in the preparation.

A. Trophozoite in early prophase—blepharoplast just divided, and flagella separating into two bundles. Fixed in osmic acid vapor. × 2750.

B. Prophase, showing new chromatic basal rod and undulating membrane growing out from daughter blepharoplast. Fixed in Schaudinn's fluid. × 1200.

C. Later prophase, showing parabasal body split almost entire length. Fixed in 1 per cent. chromic acid. × 500.

D. Late telophase, all structures duplicated, and animals ready to separate. Note flattened, twisted appearance of parabasal bodies—also the pellucid margin of the undulating membrane. Fixed in 1 per cent. chromic acid. × 1000.

E. Slightly earlier stage of telophase—nuclei still connected by paradesmose. Fixed in Flemming's strong solution. × 1300.

F. Isolated neuromotor system, dry fixed, and stained with Wright's stain. × 1150.

axostyle, after the preparation is stained, much better than others. It is much more apparent in individuals fixed in osmic-acid vapor, chromic acid, and strong Flemming's fluid, than in Schaudinn's fluid, Carnoy's fluid, and Gilson's fluid. It shows very well in specimens stained with Wright's stain. At its anterior end, where it passes under the nucleus, it expands into a capitulum at least twice its lower width, and then tapers rapidly to a point where it is connected to the centroblepharoplast. Passing from its enlarged portion, the axostyle almost invariably takes a bend of at least 90 degrees. The particles of cytoplasm surrounding the organelle at this point stain more deeply than the rest of the proximal cytoplasm (Fig. 1, *A*). The axostyle does not seem to be a cylindrical rod. It is flat, and, at its posterior end, it is sharply pointed. It has been observed in some preparations to be folded back on itself after it has pierced the body. Frequently the trunk is not parallel for its entire length but gradually enlarges from its most narrow portion directly posterior to the capitulum to its maximum width near the posterior end (Fig. 1, *A*). Then it tapers at once to an acute point. In some other cases, the width of the trunk is the same throughout its length.

The granules of the axostyle—axostylar chromidia (*Axo. chrom.*, Fig. 2)—stain intensely, and it is by their aid that the axostyle is recognized more frequently than by its own outline. They are sometimes arranged in a straight line (Fig. 3, *A*), occasionly they are arranged in spiral rows, (Fig. 1, *A*), and not infrequently one finds them scattered throughout the organelle in an apparently hit-or-miss fashion.

The literature concerning the *parabasal body* (*Par. b.*, Fig. 2) is so well reviewed in previous papers (Kofoid and Swezy, '15, Cutler, '19) that it is sufficient to state in this paper that Janicki ('11, '15), Alexieff ('13), Kuczynski ('14), Cutler ('19), and Wenrich ('21) have all reported the presence, in various trichomonads, of darkly-staining, club-shaped organelles—*in addition to* the elongated, slender, chromatoidal rod that lies under the undulating membrane. Kofoid and Swezy ('15) are inclined to regard this as the first step in the origin of the new chromatic basal rod in the prophase of fission. The figures of the developing

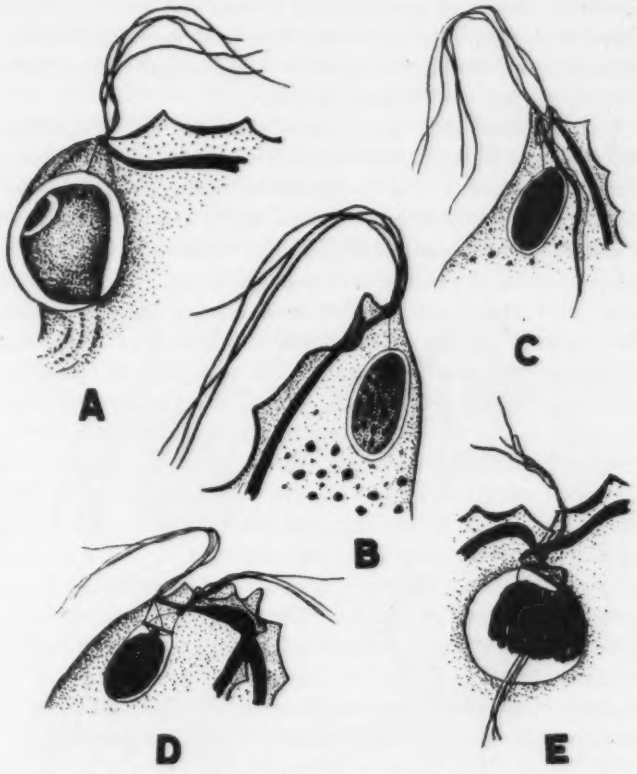

FIG. 4. These figures (Fig. 4 and Fig. 5.) are drawn to show the succession of nuclear changes during mitosis. The chromatic basal rod, undulating membrane, and anterior flagella are also indicated in order to orient the specimen. All material fixed in Schaudinn's fluid, drawn with the aid of camera lucida, and reproduced at a magnification of 2000.

A. Resting nucleus, showing karysome.

B. Chromatin beginning to condense and break up into irregular fragments that tend to arrange themselves linearly. Chromidia appearing in the cytoplasm.

C. Linear arrangement of chromatin more pronounced, centroblepharoplast has released centrosome, which has just divided, as has the blepharoplast and rhizoplast. New chromatic basal rod appearing, accompanied by thin undulating membrane. Cytoplasmic chromidia disappearing.

D. Flagella, chromatic basal rod, and undulating membrane completely duplicated; paradesmose has descended to nuclear membrane.

E. Chromosomes strung over the subparadesmosal fiber—judging from the complex appearance and probable number, they have already divided.

chromatic basal rod published by Kofoid and Swezy ('15), presumably in support of this view, differ from figures published by the other authors purporting to show parabasal bodies, both in size, shape, and spacial relationships.

The parabasal body is an elusive organelle, and is apparently dissolved out by some fixatives, leaving nothing to suggest its shape or position. It is very sensitive to decolorization, and is seldom seen in those preparations which are decolorized sufficiently to permit analysis of mitotic phenomena. But in preparations fixed with chromic acid, osmic acid vapor, or Flemming's fluid—and perhaps others—that are not too strongly decolorized, the parabasal body is a very conspicuous feature of *Trichomonas termopsidis*. If the parabasal is not carefully decolorized, it appears as a stout, club-shaped, serpentine organelle which takes its origin from the centroblepharoplast. After more critical destaining, a darker line appears, that is not always in the center, but which weaves from side to side, suggesting the probability that it does not lie within the parabasal body as is indicated by the previous writers, but is on the outside of it. This line—the chromatic filament (*Chrom. fil.* Fig. 2)—is undoubtedly the "parabasal thread" of Janicki. The body appears to have little rigidity of its own, as it sometimes loops itself back and forth on the axostyle (Fig. 1, *A*), and occasionly surrounds it. The parabasal is about the same length as the axostyle, and about the same breadth, but is otherwise readily distinguishable from it.

Mitosis.

Mitotic phenomena in *Trichomonas termopsidis* are strikingly different from any of the types described by Kofoid and Swezy ('15) for *Trichomonas*, but are identical with the type described for *Trichomitus termitidis* Kofoid and Swezy ('19). Briefly these are as follows: the nucleus darkens, and shortly thereafter the surrounding cytoplasm becomes charged with chromidia (Fig. 4, *B*) which soon disappear. Then the centroblepharoplast releases the centrosome (Fig. 3, *A*), and the remaining blepharoplast splits into two daughter blepharoplasts. Simultaneously the nuclear rhizoplast and the parabasal body split (Fig. 3, *C*). The centrosome maintains its connection with the two new blepharo-

plasts by means of rhizoplasts (Fig. 4, *C*). As the blepharoplasts become separated, one remains with the old chromatic basal rod, retaining two of the original flagella, the old undulating membrane, and one of the nuclear rhizoplasts. It completes its locomotor apparatus by growing out two new anterior flagella. The other blepharoplast takes two anterior flagella and the other nuclear rhizoplast, but immediately starts to equip itself with two new anterior flagella, and a new chromatic basal rod, complemented by a new undulating membrane (Fig. 4, *C* and Fig. 3, *B*).

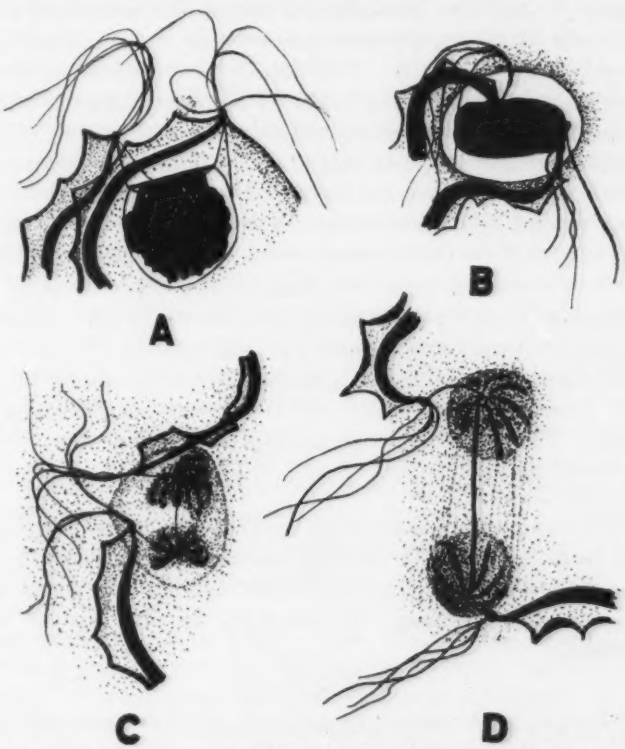

FIG. 5. *A*. Chromosomes have straightened themselves out and are seeking the polar extremities of the paradesmose—they are still on the subparadesmosal fiber.

B. Chromosomes beginning to congregate at the polar ends of the paradesmose.

C. Chromosomes grouped in rosettes at ends of paradesmose, and beginning to pull apart.

D. Chromosomes still in rosette formation, but widely separated, and ready to reorganize.

In the meanwhile the centrosome has split, and the two portions migrated apart, spinning out between them an intensely staining bar, the paradesmose. By the elongation of the rhizoplasts connecting the centrosomes and blepharoplasts, the paradesmose descends to the nuclear membrane. As it approaches the membrane, the chromatin within begins to arrange itself in linear fashion perpendicular to the paradesmose, and shortly after, the paradesmose comes in contact with the membrane, and is greeted by a projection of chromatin that is thrust up from the central mass. At this time, the subparadesmosal fiber appears (Fig. 4, *D*) upon which the chromosomes seem to arrange themselves shortly after (Fig. 4, *E*). Probably the chromosomes divide at this time while they are in this position, for the next figures we are able to find show them being gradually drawn to the ends of the paradesmose, without any indication of spindle formation. The number of chromosomes is difficult to determine, but is somewhere around ten. The chromosomes form irregular clusters or rosettes at the ends of the paradesmose, which always seems to maintain itself as a fairly straight line (Fig. 5, *C*). The two masses of chromosomes continue to migrate apart during anaphase (Fig. 5, *D*) and the paradesmose persists. In telophase, the nuclei assume their characteristic ellipsoidal shape, surrounded by hyaline zone and nuclear membrane, and the paradesmose gradually fades away. The two animals now separate by a plasmotomy, which is materially aided by the activity of the locomotor apparati of the two individuals.

The division of the axostyle we have not been able to observe—but from its capricious staining reactions during prophase, it seems probable that it may be absorbed, and new ones grown out from the blepharoplasts. By telophase, the two axostyles are complete.

Multiple Fission.

Multiple fission seems to occur rarely in *Trichomonas termopsidis*. In the material worked on, fifteen or twenty cases were observed, but they were all on one slide—that is, from the intestinal contents of one termite. There were two types of multiple fission, the one where the nuclei were in a polar position (Fig. 1, *C*), and the other where the nuclei were in a central

position (Fig. 1, *B*). These two types occurred with approximately equal frequency. In no case was the somatella composed of more than four prospective individuals, and was always found in the prophase of the second division. These somatellæ were much larger than might be suspected. The greatest and least dimensions of ten of them (nuclei in central position) averaged 80.47 micra and 48.79 micra respectively. The extremes of the greatest dimensions were 111.80 micra and 62.68 micra, and of the least dimensions, 66.91 and 30.49 micra. It is quite possible that these multiple fission figures are related to the giant race of *Trichomonas*—noted above—that was found in some of the termites examined.

Whether multiple fission represents a definite stage in the life cycle of *Trichomonas termopsidis*, or whether it is simply a chance happening stimulated by some particular complex of circumstances, we do not know. It is significant, perhaps in favor of the latter hypothesis, that multiple fission was found only in one termite.

Cysts.

Nothing was found on any preparations which could be identified with certainty as being the cysts of *Trichomonas termopsidis*. The protozoön is probably transferred from host to host in its vegetative phases.

Relationships.

This flagellate has been assigned to the genus *Trichomonas* on purely morphological grounds—that is, because of its possession of a cytostome, four anterior flagella, an undulating membrane, a chromatic basal rod, an axostyle, a parabasal body, and a centroblepharoplast.

Its nearest relatives appear to be *Trichomonas trypanoides* Duboscq and Grasse, and *Trichomitus termitidis* Kofoid and Swezy. It is difficult to state all the points of difference of *T. termopsidis* from *T. trypanoides* because of the scantiness of the description of the latter (Duboscq et Grassi '24.). But it is certain that there is a difference in size—"Les *T. Trypanoides* de courbure normale ont une taille assez constante de 16 micra." It is also found in a host, *Reticulitermes lucifugus*, which belongs to a different family (*Rhinotermitidæ*) from that of *Termopsis* (*Kalo-*

termitidæ). And finally, in *T. trypanoides*, there is a notable lack of constancy in the number of anterior flagella, which vary from one to four.

Trichomonas termopsidis differs from *Trichomitus termitidis* primarily by the possession of an axostyle. *Trichomitus termitidis* is described from *Termopsis angusticollis*? (Kofoid and Swezy, '19). In our material, we are positive of five colonies of the sixteen studied as being *Termopsis angusticollis*, but it is very probable that of the nine remaining unidentified colonies (two were identified as *Termopsis nevadensis*) some are *Termopsis angusticollis*, as the distribution and frequency of occurence of these two species is the same in Oregon and California (Banks and Snyder, '20). In mitosis, *Trichomonas termopsidis* is identical with *Trichomitus termitidis*, which differs, as far as we are aware, from every other form of trichomonad division described. Inasmuch as both forms are found in the same hosts, and as a size race of *Trichomonas termopsidis* agrees in measurements with those given for *Trichomitus termitidis*, and more particularly because the same peculiar type of phenomenon takes place at mitosis in both forms, which has not been described for any other forms, it would appear that *Trichomitus termitidis* should be suitably confirmed before its validity is established.

As *Trichomitus termitidis* differed from its type species, *T. parvus* Swezy ('15) in having the type of division where the centrosome is separated from the blepharoplast during the process, whereas division in the type species was of the kind described in *Trichomonas* and *Eutrichomastix* by Kofoid and Swezy ('15), the species of *Trichomitus* found in the termite was placed in a new subgenus, *Trichomitopsis*. Then, since *Trichomonas termopsidis* differs from the other trichomonads previously described, in the same manner, it is proposed to assign this flagellate to *Trichomonopsis* subgen. nov.

Trichomonas with centrosome separated from blepharoplast at mitosis. **Type Species,** *Trichomonas termopsidis* Cleveland, from *Termopsis nevadensis* Hagen, and *Termopsis augusticollis* Hagen.

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RECENT CONTRIBUTIONS TO THE KNOWLEDGE OF THE CRYSTALLINE STYLE OF LAMELLIBRANCHS.

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The crystalline style of pelecypod and of gastropod molluscs has long been a fascinating subject for investigation by students of these groups. In a paper (Nelson, '18) published six years ago the attempt was made to summarize and to analyse the more important contributions which had appeared up to that time, and by original observation to solve some of the problems regarding the origin, nature, and function of this structure. The conclusions of Coupin, '00, Mitra, '01, and others, that the crystalline style contains strong amylolytic ferments, were confirmed. In addition the style was shown to have at least two other functions. The first and more important of these is the rôle played by the style in the separation of food materials from sand and other waste. The powerful cilia of the style sac spin the style around on its long axis while pushing it anteriorly into the stomach. The head of the rotating style gathers the food strings coming from the œsophagus and as the whole mass is whirled around in the stomach much of the sand and other non-digestible matter is sorted out mechanically by the ciliary tracts of the stomach wall, and passed on into the intestine. At the point where the head of the revolving style comes into contact with the anterior wall of the stomach there is developed a tough resistant covering which I named the "gastric shield" (*flèche tricuspide* of Poli, 1791). The importance of such a sorting and stirring mechanism in animals in which ciliary activity has completely replaced muscular peristalsis, was pointed out.

In forms such as most of our common bivalves, where the style sac is incompletely separated from the intestine by two ridges or typhlosoles, an additional function is served. Food particles escaping from the stomach may be passed across the faces of the typhlosoles from the intestine and then be incorporated into the style. Most of such returned material enters the style sac near

its base and is built into the soft core of the style. I have found oysters in which the entire core of the style was almost a solid brown mass of *Glenodinium*. In this position food materials at first rejected from the stomach in the separation of inert materials may be recovered as the style is moved gradually forward into the stomach.

In the interval since this paper, Nelson, *l. c.*, was published there have appeared several noteworthy contributions to the knowledge of the physiology of digestion in bivalve molluscs, and of the function of the crystalline style. It is well therefore to review these at this time and to give such additional information as has been accumulated during the past seven years in order that the present status of the problem of the crystalline style may readily be ascertained.

Edmondson ('20), in a detailed and very well illustrated paper, describes the reformation of the style of *Mya arenaria* following its extraction. Among the species of bivalves which occur on the northwest coast this investigator found in *Cardium corbis*, *Saxidomus giganteus*, *S. nuttallii*, and *Paphya staminea*, that starvation or removal from the water resulted in dissolution of the style "within a few days at most." In other species, notably *Siliqua patula*, *Schizothærus nuttallii*, *Macoma nasuta*, the style was found to be far more resistant to dissolution, being present even at death through starvation. In *Mya* only slight dissolution of the style occurred even after 14 days out of water. (Contrast this with the oyster, in which dissolution of the style occurs while the molluscs are exposed during the low tide; Nelson, '18.) As Edmondson points out, the forms with resistant styles possess a style sac nearly or completely separated from the intestine.

Experiments were performed with *Mya* in which the mantle was cut in the midline along the ventral surface for a distance of 15 to 25 mm. posterior to the pedal opening. A transverse cut was made near the middle of the style sac and the style extracted. The clams were then planted out and the rate of regeneration of the style studied. No food was taken apparently until the style was sufficiently regenerated to project into the stomach, from which Edmondson concludes that ingestion and digestion of food

depend upon the degree of development of the crystalline style. A period of approximately 74 days were required for complete reformation of the style following the extraction.

This part of Edmondson's work and the conclusions he draws from it are open to some objection. As the experiments were carried out, there is no way of distinguishing the time actually required for style regeneration from the period of inactivity resulting from the effects of the operation. Out of 147 clams operated upon of which the observation period was in excess of three weeks, only 61, or approximately 42 per cent, survived the operation. Any operative procedure which results in the death of over one half of the animals should be carefully checked before conclusions are drawn from the results, and it seems that such checks were not made. The cut through the mantle alone must seriously have interfered with feeding. Bivalve molluscs are very sensitive to injury and will "sulk" without feeding for long periods after even slight disturbances.

A much better method of determining the period required for regeneration of the crystalline style would seem to be to determine the rate at which this structure is pushed forward into the stomach and dissolved during the normal feeding of the mollusc. This may be determined readily in forms which like the oyster and fresh-water mussels have a style sac incompletely separated from

FIG. 1. The crystalline style of *Lampsilis luteola* taken from an individual which had been allowed to siphon in water containing a suspension of fine carmine grains. The spiral bands are composed of carmine which was caught upon the gills, carried into the stomach, thence to the intestine from which it was fed across the typhlosoles at two points near the anterior end of the style sac and incorporated into the style.

the intestine. A weak suspension of finely divided carmine if added to a water rich in food materials will usually be ingested along with the food and may be incorporated into the style. In some instances the carmine is passed across the typhlosoles into the base of the style sac and results in a red style. In others, the carmine may be fed into the style sac at some point between the stomach and the base of the sac, in which case the carmine forms a spiral band about the style as it is rotated and pushed anteriorly, giving the effect of a barber's pole (Fig. 1). Allen ('21) records a similar observation in a fresh-water mussel where minute green organisms took the place of the carmine.[1]

Complete regeneration of the crystalline style in *Ostrea*, *Modiolus*, *Anodonta*, and *Lampsilis* will take place at summer temperatures in from approximately 15 minutes in the oyster to a few hours in the other genera. Allen ('21) finds style regeneration in fresh-water mussels in about 24 hours. It is difficult to believe that a process which occurs in at the most a few hours in many of our common bivalves should require two and a half months in an active rapidly growing mollusc such as *Mya*. Although I have had no experience with this form, it would seem possible to inject by means of a hypodermic syringe a small amount of carmine in sea water near the base of the style sac and to determine the speed at which this pigment, incorporated into the style, is carried toward the stomach. If carefully performed such an operation should be far less drastic in its effects than was the technique employed by Edmondson.

In their admirable work on the natural history and propagation of fresh-water mussels Coker and his associates ('21) accept the conclusions of my 1918 paper, but contribute no original observations regarding the function of the crystalline style. Included in the former paper, pp. 88–91, are the observations of Dr. Franz

[1] While this paper was in press I received a copy of the report of Dr. J. H. Orton '24 on the causes of unusual mortality among oysters on English oyster beds; Ministry of Agriculture and Fisheries, London. On page 54 of this report is figured the crystalline style of *Ostrea edulis*, bearing a spiral band of food organisms. In a footnote on the same page this author suggests that one function of the style is "the mechanical one of drawing the mucous strings enveloping the food material into the stomach by twisting the strings around the shredded revolving head of the style like a capstan." In my 1918 paper, pg. 101, it is observed that "so strong is the tractive force of the rotating style that strings of mucus from any part of the body if led to the stomach cavity, are at once drawn in and wound up in the food mass."

Schrader on the food of mussels. This investigator found that only about one half of the diatoms and green algæ taken in were digested, and he concluded that these organisms play a comparatively unimportant rôle in the food supply of mussels. He apparently was unaware that in the process of sorting over of the acquired food materials by the ciliary tracts of the stomach, aided by the rotation of the crystalline style, many food organisms are shunted off into the intestine along with the sand and dirt. Especially may this be true of relatively heavy forms such as the diatoms. Some of these escaped food organisms may be passed across the typhlosole into the style sac and thus eventually be returned to the stomach, but a considerable number escape undigested from the anus. Blegvad '15 likewise draws false deductions as to the "insignificant" rôle played by plankton organisms in nutrition, from the finding of living planktonts in the fæces of the European oyster.

While at Madison, Wisconsin, I kept *Anodonta* and *Lampsilis* for months at a time in clear running water in which the chief food supply consisted of desmids, diatoms, and nannoplankton forms most of which were growing upon the sides of the tank and upon the shells of the mussels themselves (Allen, '14). Examination of the intestinal contents revealed many living organisms but also the empty tests of numerous diatoms which had been digested. Imperfect as may be the mode of separation of food from dirt by the ciliary mechanisms within the alimentary canal of bivalves, the wonder is that it functions as efficiently as it does. Such living organisms as are cast out in the fæces are not altogether lost since they accumulate on the shells or upon nearby objects, where they multiply rapidly within the fæcal remains and form a rich growth which is continually contributing its quota of food to the siphons of the mollusc (Allen, '14; Martin, '23).

Allen ('21) gives a detailed account of experiments on the effects of various food organisms in the formation of the crystalline style of fresh-water mussels. The details of this work cannot be discussed here, but in general they confirm and extend the findings of his preliminary paper (Allen, '14) and my own conclusions (Nelson, '18) regarding the rôle of food in style regeneration. He further showed that nannoplankton is relatively more effective than is net plankton in effecting style formation.

The only essential difference between Allen's results and mine is that in fresh-water mussels he found that relatively little escaped food was fed back into the stomach by way of the style, and he concluded that this reclaiming function of the style was of relatively little importance in these animals. The largest amounts of such material were found in the core of the style soon after regeneration of a new style had begun, an observation which I can confirm in *Anodonta grandis*. The relative efficiency of this retrieving mechanism in various bivalves will have to be extensively studied before we shall be in a position to determine its importance to molluscs generally. The fact that *Mya*, *Teredo*, *Martesia* and many other genera with a style sac nearly or quite separated from the intestine are able to exist without such a mechanism indicates that on the whole it plays perhaps a minor rôle in nutrition. Phylogenetically it probably represents the development of a strong recurrent tract of cilia situated in the posterior part of the stomach of the ancestors of present-day types; such a group of cilia as may, for example, be demonstrated in the stomach of the larval oyster.

Allen (*l. c.*) lays still further emphasis upon his conclusion of 1914, voiced likewise in my 1918 paper, that the crystalline style arises in response to the presence of food in the stomach. Evidence that such may not be the case is discussed further on in connection with the findings of Berkeley ('23).

Nogouchi '21 examined the crystalline styles of various marine bivalves and gastropods for *Cristispira*, a large active spirochæte which was first discovered in the style of the oyster. The spirochætes were found most frequently in *Ostrea*, next in *Mya*, then in *Modiolus;* but not at all in *Venus*, *Ensis*, *Mactra*, *Mytilus*, *Pecten*, *Fulgur*, and *Nassa*. Gross, however, has reported *Cristispira pectenis* from the crystalline style of *Pecten*. Nogouchi did not know of my 1918 paper in which is discussed briefly the occurrence of *Cristispira* in the styles of certain bivalves and its absence from others. Nogouchi observed that the style of the oyster quickly liquefied after extraction from the body, and that only oysters freshly removed from natural conditions contained this structure. He believes that the great abundance of *C. balbiani* in the oyster is due to the relatively soft consistency of

the style in this mollusc, and that the absence of spirochætes in many molluscs is due to the very firm and resistant styles which they possess.

Although the solidity and resistance to dissolution of the style may effect the distribution of *Cristispira*, they are certainly not the most important factors. As Nogouchi himself admits, and as Edmondson showed, the style of *Mya* is very firm and relatively resistant to dissolution, yet it harbors *Cristispira* in an abundance second only to that of *Ostrea*.

Martin ('23), studying the relative importance of the net plankton and of nannoplankton in the food of the oyster, found that water from which even the smallest nannoplankton organisms including bacteria had been removed would, if well aërated, permit reformation of the style in *Ostrea*. He concluded that although the appearance of the style in this mollusc is usually correlated with the taking in of food, this structure may arise in the complete absence of food, presumably as a response to the act of siphoning.[1]

Three months after the publication of Martin's paper appeared an interesting communication from Berkeley ('23) regarding the function of the crystalline style as a possible factor in the anaërobic respiration of certain marine molluscs. This investigator attempts to account for the continued production of carbon dioxide by marine molluscs kept under anaërobic conditions, as demonstrated by Collip ('21). In seeking a possible explanation of this production of carbon dioxide Berkeley tested the reactions of various tissues and of the style of *Saxidomus* with an alcoholic solution of gum guaiacum. Only in the case of the style did he obtain a deep blue color, which suggested to him that this structure might be associated with anaërobic respiration. Molluscs kept under anaërobic conditions showed in all cases an absence of the style. In an earlier paper (Berkeley, '21) it was shown that a disappearance of glycogen accompanies anaërobiosis in *Saxidomus gigantea*, though not in *Paphia* nor in *Mya*.

It is pointed out in a footnote of Berkeley's ('23) paper that the work was done in ignorance of the publications of Mitra, Allen,

[1] Although an oyster has no siphons, this term has come into such general use for the process of passing water through the gills of a mollusc that it seems best to employ it here—especially as no good substitute appears to be available.

Nelson, and Edmondson, and that in the light of the findings of these and of other workers a more critical series of experiments is needed to determine the relative importance of food and of oxygen in determining style formation. Berkeley concludes that the presence or absence of the style depends upon the presence or absence of oxygen, and he makes the astonishing assumption that food plays no part in the building up of this structure, on the ground that oatmeal added to the water caused no regeneration of the style. No examinations were made to determine whether the animals were eating the oatmeal, nor were any tests made using the natural plankton food of the molluscs.

Taking the results of Martin and Berkeley together, however, I believe that Allen and I were mistaken in laying undue emphasis upon the rôle of food in stimulating style secretion. Although the presence of food in the stomach may play a part in the mechanism of style formation, Edmondson's finding that no food was taken by *Mya* until after the head of the regenerating style protruded into the stomach; Martin's results in obtaining style regeneration in aërated water devoid of all net and nannoplankton; and Berkeley's conclusion that no style forms under anaërobic conditions, all point to the probability that secretion of the crystalline style may be a direct response to siphoning, regardless of whether the incoming water contains food organisms or not.[1]

The chief criticism centers about the following conclusions of Berkeley; first, his assumption that since the style disappears when the bivalve is kept under anaërobic conditions it therefore represents a reserve of oxygen. As well might one conclude that all secretions contain reserve oxygen since secretion is diminished during decreased activity of the organism. Second, no correlation was shown between the size and persistence of the crystalline style on the one hand and the degree of aëration of the environment on the other. As bearing upon the first of these assumptions Gray, '23, showed that for ciliary movement the degree of mechanical activity exactly parallels the relative amount of oxygen absorbed. Since the formation and movement of the

[1] Orton, '24, l.c., pg. 55, observes that sound *O. edulis* will reform a style in the absence of food, but from the text it is not clear that all nannoplankton was removed from the water.

style are so largely dependent upon the activity of the powerful cilia of the style sac, it is to be expected that no style would form in an absence of oxygen. From what we know also regarding the oxygen consumption of the glands of mammals it may be assumed that relatively little secretion of style substance would occur in an absence of oxygen. Berkeley's work is open also to the objection that nowhere does he mention having watched his molluscs to determine whether they opened up and siphoned in the anaërobic water. Such observations are imperative owing to "sulking" on the part of some bivalves after handling, even when the surrounding water is plentifully supplied with oxygen and food. I find that the oyster will not open in oxygen-free water until it becomes too weak to remain closed. Dissolution of the style occurs when many species of bivalves remain tightly closed for a few hours, irrespective of whether the surrounding water is aërated.

In connection with the relation between the degree of aëration of natural waters and style formation it is of interest to compare the crystalline style of *Pisidium idahoense* with that of *Mactra*. *Pisidium* occurs in abundance in the mud at the bottom of Lake Mendota, Wisconsin, where for two thirds of the year the water may be completely devoid of oxygen (Birge and Juday, '11, Cole, '21). *Mactra* on the other hand lives in or close to the breaker line along sandy coasts, where the water at all seasons of the year is saturated with oxygen. The style of *Pisidium* is no larger than that of other Cyrenidæ which live in relatively well aërated creeks and ponds, whereas the crystalline styles of all *Mactra* which I have examined are relatively large, firm, and among the best developed of the styles I have found in any bivalve.

In *Ostrea* the direct relationship of crystalline style secretion to feeding can readily be demonstrated since this process assumes a somewhat rhythmic character. During the flood tide when the bivalves are feeding actively the style is large and firm, but by the late ebb tide, at which time most of the sand has been sorted out and removed from the stomach and digestion is well under way, the style may be reduced to a soft amorphous mass of jelly. The crystalline style is usually thin or entirely lacking at sunrise before active feeding of the molluscs has commenced. I agree

therefore with Berkeley that his conception of the respiratory function of the crystalline style rests upon insecure evidence; and I believe that this theory, interesting though it be, must be added to the long list of the purely suppositious functions of the style which have been proposed during the past two hundred and thirty years.

Bökmann ('23) figures the crystalline style and the style sac of *Mytilus chorus* and his account agrees with the conclusions of List and of Mitra. From a histological study of the ciliated epithelium of the style sac he concluded that this must serve to put the style in rotation, although he did not observe the movement. This author apparently was not familiar with the work of recent American investigators.

A paper of much interest and valuable for its collection of many observations under one head is that of Yonge ('23) in which for the first time is given in one place an adequate account of the mechanisms of feeding and of digestion in a bivalve mollusc. This investigator traces the fate of food particles from the time they enter the incurrent siphon until the waste is expelled from the anus. No new information regarding the function of the style is given in this paper, but the recent literature is well summarized and a clear description is given of the rôle of the crystalline style in digestion in *Mya*. A study is made of the effects of varying concentrations of enzyme and of substrate, using the style and starch solutions. The optimum temperature for the reaction was found to lie at approximately 32° C., with complete destruction of the enzyme at 51° C. From the data thus collected it is concluded that the powerful amylolytic ferment of the style of *Mya* shows all of the characteristic properties of such an enzyme.

It is pointed out that the lamellibranchs may be arranged into taxonomic groups on the basis of the morphology of the style sac and intestine, but that such a grouping does not follow the modern classification based on the gill structure. Yonge considers that either our present system of classification is faulty or that independent evolution has occurred within the digestive system. I believe the latter to be the case since the morphology of the digestive tract is to a high degree correlated with the food

habits and the habitat of the molluscs. This aspect of the problem is discussed likewise by Robson ('22) for the style sac and intestine of gastropods as well as of lamellibranchs.

Criticisms of Yonge's work are, first, his failure to consider the important rôle of the leucocytes in digestion, especially of fat;[1] and second, an inadequate appreciation of the part played by the hepatopancreas in the digestion and absorption of food as demonstrated by List ('02). From microscopic examinations of this organ during the later stages of digestion in *Ostrea* and especially in *Modiolus* I am led to believe that in these forms even more food is digested in the hepatopancreas than in the stomach. I believe the functions of the stomach, in some lamellibranchs at least, to be chiefly those of sorting over of food materials and the final separation of dirt, and of mixing food and enzymes, rather than those of an organ where complete digestion is effected.

Lazier ('24) in a valuable paper on the morphology of the digestive tract of *Teredo navalis* confirms for *Teredo* the conclusions of my 1918 paper regarding the origin and function of the crystalline style. His investigation of the morphology of the stomach, style-sac, and intestine shows that these structures are essentially similar to those of other molluscs in which the style-sac is completely separated from the intestine. The style of *Teredo* is believed to be rotated in the manner I described for *Modiolus*, and in *Anodonta*, although the actual rotation was no, observed by Lazier.

In some of the marine borers, however, (*Pholas* and *Martesia*t for example) the style is of such proportions that if rotation occurs it must be very slow. In my 1918 paper is figured a transverse section of *Martesia* showing a style of a mass approximately equivalent to the entire remainder of the body exclusive of the gills. This aspect of the problem needs further investigation, especially as to the part played by the style in the acquisition and possible sorting over of the borings ingested by *Teredo* and its wood-boring allies.

Churchill and Lewis ('24) make a valuable contribution to our knowledge of the mechanism of feeding in young bivalves. No

[1] A recent paper by Vonk '24 gives a good summary of the work in this field and adds some original observations.

new data are given regarding the nature of use of the crystalline style, the authors accepting the conclusions of my 1918 paper.

In conclusion it is apparent that the main facts regarding the origin, nature, and function of the crystalline style may be considered as quite firmly established and that we are now in a position to attack with the aid of modern methods and the newer knowledge of general physiology some of the problems of nutrition in molluscs.

Summary.

The period of 74 days required for style reformation in *Mya* as determined by Edmondson ('20) represents not only the time during which a new style is regenerating after extraction, but also the period of recovery following the rather drastic operation performed by this investigator. From data procured from other species of lamellibranchs it would appear that the time necessary for actual reformation of the style is much less than the figure given.

It was shown (Nelson, '18) that owing to the imperfect mode of separation of food particles from dirt and sand in the stomach of bivalve molluscs, some undigested food materials may escape down the intestine. In those forms in which the style sac is incompletely separated from the intestine a part of this rejected food material may be incorporated into the crystalline style, thus eventually being returned to the stomach. The remainder pass out undigested in the fæces. Failure to recognize this fact has led at least two recent investigators to conclude that such living organisms as are recovered from the fæces cannot be utilized by the molluscs as food.

The degree of solidity and of resistance to dissolution of the crystalline style are suggested by Nogouchi ('21) as factors controlling the presence or absence of Cristispira. That the solidity and the resistance of the style are not the primary factors involved in the distribution of Cristispira is shown by the presence of these spirochætes in the styles of *Ostrea* and of *Mya* which represent respectively minimum and maximum hardness.

Martin ('23) showed that a style may arise in *Ostrea* as a direct response to siphoning in aërated water even when the incoming water is devoid of all net and nannoplankton or other sources of

food. Berkeley ('23) found no style reformation under anaërobic conditions. His conclusion, however, that the style represents a reserve of oxygen is open to serious objection as herein explained.

The conclusions of Edmondson, '20, Allen, '21, Coker and others, '21, Bökman, '23, Churchill and Lewis, '24, and of Lazier, '24, agree in confirming the work of Coupin, '00, and the conclusions of my 1918 paper. Except in so far as the latter are modified by the findings of Martin and of Berkeley, as set forth in this paper, it is believed that the conclusions of Coupin and of the writer represent the consensus of opinion today regarding the origin, nature, and function of the crystalline style of lamellibranch molluscs.

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THE CONDITIONS OF ACTIVATION OF UNFERTILIZED STARFISH EGGS BY THE ELECTRIC CURRENT.

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While sensitivity to the electric current seems universal in living matter, its degree varies greatly—apparently in correspondence with the wide variation in general irritability. It is most highly developed in rapidly responding tissues such as muscle and nerve; but it can be shown to exist in supposedly insensitive cells like epidermal cells, which respond to electrical stimulation by increase of conductivity.[1] Specialized sensory receptors (retina, auditory or chemical senses) all respond to the electric current as well as to their own appropriate forms of stimulation. Electrical sensitivity thus appears to be the primary form of sensitivity.[2] In general, as the work of Nernst and his successors has shown, it is intimately connected with polarizability, which is dependent on the presence of diffusion-resisting or semipermeable partitions enclosing or pervading the protoplasmic system. Evidently the electric current acts within the living system by influencing the chemical reactions at its polarizable surfaces, as in electrode action in general; in living protoplasm the surfaces concerned are those of the protoplasmic structures, and especially of the films delimiting or separating the protoplasmic phases.

The directive or stimulating action of the electric current on growth has been demonstrated in a number of cases, although much remains to be done in this field. The unfertilized egg-cell, however, seems usually to be relatively insensitive to the current. The earlier evidence of electrical parthenogenesis in the eggs of marine animals is inconciusive. Schücking claims to have activated starfish eggs by passing the current from two chromic acid

[1] Ebbecke, U., *Arch. ges. Physiol.*, 1922, CXCV., pp. 300, 324.

[2] For a fuller discussion *cf.* the recent volume of R. S. Lillie, "Protoplasmic Action and Nervous Action," University of Chicago Press, 1923, pp. 273 *et seq.*

elements for one to two minutes;[1] but the conditions (nature of electrodes, distance apart, quantity of sea-water, etc.) are not described. He does not consider the possibility that acid or alkali electrolytically produced, or heat, rather than the electric current as such, may have been responsible for the effect; and in the light of our own experiments it seems highly probable that this was the case. Schücking found induction shocks to be ineffective, and we have repeated and confirmed this observation. In the condenser-like arrangement used by Delage,[2] the actual physical conditions were ill-defined. Delage's aim was to affect the eggs electrostatically (by induction); they were placed in a layer of sea-water separated by a thin sheet of mica from a sheet of tinfoil, the sea-water and the metal being connected with the poles of a battery. His results were irregular, and he himself expresses doubt as to the real nature of the conditions. The presence of currents and of products of electrolysis seems not to have been excluded.

In the eggs of amphibia McClendon was able to start cleavage and development by passing the alternating current from the lighting circuit (110 volts, 60 cycles) through the water containing the eggs.[3] In this case a current of considerable intensity acted for a brief period (1 to 2 seconds), and the effect was probably not caused by electrolytic products or heat. Further and more precise investigation of the conditions of electrical parthenogenesis in these eggs seems desirable.

Our aim in the experiments described in this paper has been (1) to ascertain whether in fact activation of starfish eggs by the direct electric current is possible, and to what degree, and (2) to determine more precisely the conditions, more particularly of current intensity, time of exposure and temperature, under which the effect is produced. The experiments were performed on the unfertilized eggs of *Asterias forbesii* at Woods Hole during the summers of 1922 and 1923. The direct current was used in all cases. Usually the eggs were exposed to the current during the interval between the breakdown of the germinal vesicle and the separation of the first polar body (prematuration period);[4] for

[1] Schücking, A., *Arch. ges. Physiol.*, 1903, XCVII., 86.

[2] Delage, Y., *Arch. zool. expér. et gén.*, Sér. 4, 1908, IX., p. xxx.

[3] McClendon, J. F., *Amer. Jour. Physiol.*, 1912, XXIX., p. 299.

[4] The period most favorable for fertilization and artificial activation.

comparison a number of observations were made with fully mature eggs. The procedure was simple; the current was passed through a shallow layer of sea-water containing the eggs, and at regular intervals portions of eggs were transferred to dishes or watch-glasses containing sea-water. Afterwards they were examined. Fertilized and unfertilized controls were kept in all cases.

Our preliminary experiments with battery currents of moderate intensity (one to twelve storage cells) gave uniformly negative results, and in all of our later experiments we used the current from the direct current generator of the laboratory. We were not able at first to find suitable electrodes. The ordinary forms of non-polarizable electrodes ("boot" electrodes) proved unsatisfactory because of high resistance and the diffusion of $ZnSO_4$ into the sea-water. When platinum electrodes were used the eggs showed partial activation (membrane-formation) in some experiments; but it could be shown (by first passing the current through a layer of sea-water and then placing the eggs in the sea-water without the current) that this effect was due to—or at least could be produced by—the products of electrolysis. These results indicated the need for an arrangement by which strong currents could be passed through the layer of sea-water without contaminating the latter by the electrode solution or products of electrolysis; and after a number of preliminary experiments the following method was devised. The wires from the direct current line (110 volts) were connected with two broad zinc plates each immersed in a dish of saturated $ZnSO_4$; these dishes with the plates constituted non-polarizable electrodes of low resistance. A rectangular glass vessel containing the sea-water in which the eggs were to be exposed was placed between the dishes. In the experiments of each summer a single vessel was used throughout the whole series; the dimensions in 1922 were 14 x 5.5 x 3.5 cm., in 1923, 13.1 x 6.6 x 3.5 cm. The depth of sea-water was kept constant as nearly as possible throughout each series, 0.4 cm. in 1922 and 0.5 cm. in 1923; the sectional area (from which the estimates of current density were made) was thus 2.2 sq. cm. in 1922 and 3.3 sq. cm. in 1923. The current was conveyed through the sea-water by massive bridges of agar jelly of the same width

as the rectangular vessel and connecting the latter with the electrode dishes. These bridges were made as follows: A concentrated solution of agar-agar in sea-water was allowed to solidify in a large beaker; the mass of jelly was then removed and cut into two blocks of the general shape indicated in the figure; one end of each block stood in the $ZnSO_4$ solution and the other in the sea-water, as shown diagrammatically in the longitudinal section (Fig. 1). By means of this arrangement strong currents (up to 2

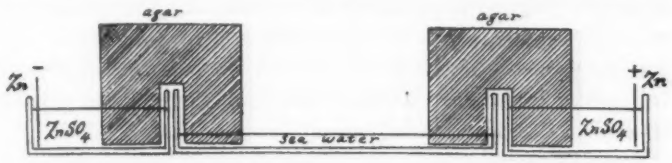


FIG. 1.

or more amperes) could be passed through the sea-water. Experiment showed that the composition of the sea-water was not appreciably affected during the flow of the current for the period of an experiment. The temperature, however, rose rapidly unless controlled. In part of our experiments the control of temperature was effected by setting the whole system in a pan of ice water; this method proved satisfactory with currents of moderate intensity (up to 150 ma./cm.).[1] In a number of experiments with stronger currents another method was used, to be described below. The strength of the current was regulated by two rheostats and measured directly by a Weston voltmeter provided with shunts so as to read as a milliammeter over the several ranges required. Intensities as high as 3 or 4 amperes were used for a brief period in some of the experiments with running sea-water described below (densities up to *ca.* 800 ma./cm.).

The usual procedure was as follows: A somewhat small quantity of eggs was placed with a pipette in the rectangular dish midway between the agar bridges (2 to 4 cm. from each). When the eggs had settled the circuit was closed, and at stated intervals, usually 2, 4, 8 and 12 minutes, successive portions of eggs were transferred to small dishes (usually Syracuse watch glasses or stender dishes)

[1] Milliamperes per square centimeter of sectional area. The commonly used unit of current-density, δ (microampere, *i.e.*, .001 milliampere, per square millimeter), is one tenth of this unit.

containing sea-water; these were kept covered except at times of examination, and the sea-water was changed several times. With small numbers of eggs this method is satisfactory and convenient. In each experiment the temperature of the sea-water in the rectangular dish was recorded at the end of the longest exposure. The temperature was measured by a thermometer with the bulb placed near the eggs. A difficulty with this method is that the bulb was incompletely immersed when the layer of sea-water was shallow, as in most of our experiments; this was especially true of the earlier experiments, where the readings were too low and a correction of 1 to 3 degrees was found necessary; with later experiments a small thermometer with a short bulb was used which gave reliable readings.

A large number of experiments (more than 50) were performed in which the vessel containing the eggs was immersed in ice water as described above; the current densities used ranged from less than 100 to 318 ma./cm. The general results of these experiments may be summarized as follows.

With currents of densities ranging from 136 to 242 ma./cm., flowing from 2 to 12 minutes, activation was either absent or negligible, provided the temperature remained below 29°. In all cases where the temperature rose to 30° or higher a variable degree of activation, usually incomplete, was obtained; and in some cases a considerable proportion of eggs developed to the swimming blastula stage. In the experiment showing the most striking effect of this kind (Aug. 22, 1922) a very typical picture of heat activation was presented; after 4 minutes exposure to a current of 227 ma./cm., only a few eggs (*ca.* 3 per cent.) formed membranes; with exposures of 8 and 12 minutes almost all formed membranes and a large proportion formed blastulæ (*ca.* 75 per cent. with 8 minutes and 25 per cent. with 12 minutes). The temperature at the end of the maximum period of exposure (12 m.), allowing for the error of measurement, was 30° or over. In such a case the activation caused by the current is mainly if not entirely an effect of the high temperature, and not of the current as such. This is shown by the fact that activation was never produced by the same current at lower temperatures (28° or ower); also by control experiments in which eggs were activated

by sea-water which had been warmed to 30° or higher by the current, the latter being shut off before the eggs were introduced.

It seems probable, nevertheless, that a part of the activating effect observed in this and similar experiments is to be attributed to the current; *i.e.*, that there is a summation of the effects of heat and current, since the degree of activation was greater than would usually be produced by exposure to a temperature of 30° for the periods used. According to earlier observations, activation by warm sea-water (acting alone) requires a temperature of at least 29°, and at 30° few eggs develop to a blastula stage after less than 15 minutes' exposure.[1] In other words, the effect of high temperature appears to be greater when a current is flowing through the sea-water containing the eggs than when no current is flowing. We have not, however, performed definite controlled experiments to determine with exactitude the degree of this additive effect. An analogous phenomenon is seen in the activation of starfish eggs by fatty acid; at temperatures of 26° and higher the effective times of exposure to the acid are much shorter than can be accounted for by the temperature cœfficient of acid activation (Q_{10} = *ca.* 3.0) shown at lower temperatures.[2] Apparently in warm sea-water the action of the fatty acid is accelerated by some condition dependent on temperature; *i.e.*, there is a superposition of acid activation upon an incipient heat activation. Similarly, in the experiments with strong currents an effect resulting from the action of the current as such appears to be superposed upon that of the high temperature.

With current-densities higher than 240 ma./cm. the difficulty of evading the temperature effect was such that it was necessary to devise another means of compensating the heating action of the current. We therefore tried exposing the eggs to strong currents in *running* instead of stationary sea-water, and after some preliminary experimentation adopted the following procedure: In place of the rectangular glass dish a paper box of the same dimensions was used. This was reinforced and made an electric non-conductor by several coatings of paraffin. A rectangular slit (*ca.* 6 x 1.5 cm.) was cut at the base of this box along one of the longer sides. A small rectangular cloth basket (*ca.* 3 x 3 x 4 cm.),

[1] Lillie, R. S., BIOL. BULL., 1915, XXVIII., p. 260; *cf.* Table II., p. 269.
[2] Lillie, R. S., BIOL. BULL., 1917, XXXII., p. 131; *cf.* Table II., p. 142.

containing the eggs, was inserted between the two agar bridges at the center of the paper-paraffin dish. This basket consisted of a frame of slender wooden sticks reinforced with thread and paraffin, to which silk bolting cloth was sewn. The bolting cloth had 200 threads to the inch—thus confining the eggs, yet allowing a free circulation of water. The apparatus was set on a wooden block in a large pan. A stream of sea water of considerable force was directed through a glass nozzle against the bottom of the paper-paraffin vessel on the opposite side of the basket from the slit. In this way a swift stream of water through the basket was obtained. After passing through the basket the water ran out of the slit down the side of the block into the pan where it was removed with a siphon. A small thermometer was p'aced with its bulb resting on the bottom of the basket.

In carrying out these experiments the usual procedure was as follows. The sea water and the current were started and the temperature was allowed to reach its position of equilibrium. Then with a pipette a quantity of eggs was placed in the cloth basket, and at the stated intervals the portions were removed to stender dishes of sea water for observation.

The density of current was estimated from the ammeter reading and the average depth of the layer of running water in the paraffined vessel. Under these conditions, even with densities so high as 600 to 800 ma./cm., the current could be passed for several minutes without raising the temperature above 29°, and with lower densities the temperature showed little increase over that of the sea water without the current. In one of the experiments with a strong current (ammeter reading 680–790 ma./cm.) passed for four minutes, the temperature reading was 29° for most of the period of flow but reached 30°–31° for a few seconds toward the end. In a second similar experiment, with a range of 648–810 ma./cm., in which eggs were removed to normal sea water at intervals of ½, 1, 1½ and 2 minutes, the temperature reading was unfortunately lost; probably 30°–31° was reached in this case also.

With such strong currents the eggs showed marked deformation during the period of exposure, adopting shapes of the kind shown in Fig. 2. This effect is temporary; within a few minutes after

removal from the current the eggs return to their original shape. Such eggs show a separation of fertilization-membranes in a considerable proportion of cases, but not in all. In the experiment just cited *ca.* 40 per cent. of the eggs exposed to the strong

FIG. 2.

current for 1½ and 2 minutes showed well separated membranes and a small proportion developed to the blastula stage. The majority underwent disintegration inside the membrane without development.

Of twelve experiments performed with this method (with good controls), using currents of densities ranging between 130 and 810 ma./cm., seven showed a varying degree of activation. Little or no activation was obtained with currents of less than 300 ma./cm. With higher intensities membrane-formation and activation occurred in a minority of eggs; the strongest currents in addition to deforming the eggs temporarily in the manner just described had a marked destructive effect.[1]

In general the results of the foregoing experiments indicate that the electric current has little activating effect upon the starfish egg unless the intensities employed are sufficient to produce well marked structural changes in the egg-system. The evidence of these changes is deformation and subsequent breakdown of most eggs. A certain proportion of eggs, however, recover and show the usual phenomena of partial activation. These effects cannot be referred to the observed rise of temperature which produces no such definite deformation. Moreover, the highest temperatures reached (30°–31°) require a much longer period of exposure for activation of the degree observed.

It should be remembered in considering the results of such experiments that the physical conditions are far from constant, and that the records of both temperature and current are subject

[1] This destructive action of strong currents on egg cells has been noted by other observers; *cf.* the case of *Crepidula* as described by Conklin, *Jour. Acad. Nat. Sciences*, Philadelphia, 1912, XV., p. 521.

to an error which may be considerable. In general the recorded current density is greater than that to which the eggs were actually exposed, since the current lines extend outside of the rectangular vessel into the overflow. There is also an error of measurement resulting from variability in the depth and contour of the layer of water between the agar bridges; this condition makes possible only an approximate estimate of the sectional areas. The usual procedure was to measure the depth of the water in the basket and on both sides of it and to calculate the cross section from these measurements. But even during a single 5-minute experiment the water level often varied considerably, sometimes because the eggs themselves clogged the silk bolting cloth thus raising the level of water inside the basket. Again, with regard to temperature, although the bulb of the thermometer was completely immersed the irregularity of the water stream is a source of uncertainty. Local temperatures may rise higher than the average temperature recorded by the thermometer; or the instrument may be actually registering the temperature of a stream of water of greater or less velocity than that to which the eggs are actually exposed. It may reasonably be assumed, however, that on the whole the errors in opposite directions compensate each other.

It was thought possible that the physiological effect of the current might be changed (increased or decreased) by changing the balance of salts in the medium. This occurs, for example, in the electrical stimulation of muscle.[1] Eggs were suspended in pure isotonic NaCl solution (0.54 *m*), washed in this solution by gentle centrifuging and decantation, and exposed to the current (densities from 139 to 262 ma./cm.) in the rectangular glass dishes under the conditions already described. A certain degree of activation results from the action of the pure NaCl solution in the absence of the current.[2] The result of passing the current through the NaCl solution containing the eggs was, however, essentially negative; four out of eleven experiments showed a slight increase in activation over that produced by the solution alone, five showed no difference, while two showed a decrease.

[1] *Cf.* the observations of K. Lucas and G. W. Mines on the electrical stimulation of muscle in *Journ. Physiol.*, 1908, XXXVII., p. 459.

[2] Lillie, R. S., *Amer. Journ. Physiol.*, 1911, XXVII., p. 289.

The combined action of the NaCl solution and the current thus shows no significant difference from that of the pure solution alone. The increase noted in the first four experiments was probably the result of a slight rise of temperature; this increases the activating effect of the NaCl solution, as control experiments showed.

Decreasing the conductivity of the medium by adding isotonic sugar solution to the sea water was also found not to alter the effect of the current on the eggs.

In conclusion brief mention should be made of similar experiments with *Arbacia* eggs. The results of these experiments were mainly negative. Little or no effect was produced by exposing the eggs in standing sea water to current-densities varying from 7.5 to 210 ma./cm. No membranes were formed and no cleavage resulted. Eggs exposed to the current and immediately afterwards treated with hypertonic sea water showed no constant increase in the percentage of activation, above eggs treated with hypertonic sea water alone. The effect of exposing to currents of high density in running sea water was also essentially negative, although some cytolysis was caused by the longer exposures. In general the *Arbacia* egg is more resistant to the current than the *Asterias* egg; this difference is probably to be correlated with the greater impermeability of the surface layer to water [1] (and presumably to water soluble substances) and its greater resistance to alteration and the action of parthenogenetic agents in general.

Summary.

1. A new type of non-polarizable ($Zn–ZnSO_4$) electrode of low resistance is described by which strong electric currents (up to 2 amperes or more) can be passed for prolonged periods through a small quantity of sea-water without appreciably affecting its composition.

2. It was found that unfertilized starfish eggs can be readily and completely activated by moderate currents, of the density 200–300 milliamperes per square centimeter; but that the effect in such cases is due almost entirely to the heating action of the current on the sea-water. When the temperature is kept below 29° such currents produce little or no effect upon the eggs.

[1] Lillie, R. S., *Amer. Journ. Physiol.*, 1918, XLV., p. 406; *cf.* p. 420.

Unfertilized eggs are thus insensitive to the current as compared with most irritable or active cells of other kinds.

3. When the eggs are exposed in a stream of running sea-water to stronger currents (600–800 ma./cm.) for brief periods ($\frac{1}{2}$ to 2 minutes), definite effects are produced. The eggs undergo marked deformation during the passage of the current, and a variable proportion afterwards show fertilization-membranes and partial activation. Complete activation of a large proportion of eggs was not possible in our experiments, although a few developed to the blastula stage.

4. We conclude that the unfertilized starfish egg is insensitive to currents of moderate intensity, and exhibits activation only when currents are used of such intensity as to produce definite and well marked structural changes in the egg-system.

5. Similar exposure of unfertilized sea-urchin eggs (*Arbacia*) to strong currents, with and without after-treatment with hypertonic sea-water, gave inconstant or negative results.

AN EXPERIMENTAL ANALYSIS OF ASYMMETRY IN THE STARFISH, *PATIRIA MINIATA*

H. H. NEWMAN.

(From the Hull Zoölogical Laboratory, the University of Chicago.)

I. Introduction.

The present paper gives the results of a portion of the experimental work carried on at the Hopkins Marine Station of Leland Stanford University in the spring of 1923. The program of work was a continuation and an extension of the experiments begun in the same laboratory in 1920 and published during the next two years (Newman, '21,*a*; '21,*b*; '21,*c*; '22).

It became apparent in 1920 that not only twinning but other developmental aberrations could be induced by retarding the rate of development of eggs and embryos in a considerable variety of ways. Of all the agents used by various investigators for retarding development, that of low temperatures seemed to be the least open to objection and is perhaps the most readily managed. It was, therefore, with the program in mind, of subjecting *Patiria* and other echinoderms to various degrees of low temperatures at various stages of development and to observe and analyse the resulting developmental aberrations, that work was begun early in April, 1923.

During some of the very first of the preliminary experiments it was found that subjection of *Patiria* blastulæ to temperatures of about 2° C. for two or three hours brought about a marked increase in the percentage of larvæ showing reversed or right-handed asymmetry. For over two months after this discovery it became a major consideration to gather an adequate mass of data on this subject and to work out the implications involved.

Before it was possible to arrive at any justifiable conclusions as to the affects of low temperatures upon asymmetry it was necessary to study in some detail the normal development of *Patiria*. It seems likely that entirely normal development is unattainable under laboratory conditions, yet the culture methods used were experimentally determined to be a vast improvement upon those

commonly adopted by experimentors upon like material. As an introduction to the experimental part of this paper we shall ask the reader to make with us a brief survey of the essential features of the normal embryonic and larval periods of Patiria, paying chief attention to the origin and development of asymmetry.

II. The Normal Development of *Patiria*.

Methods of Securing and Handling Eggs and Larvæ.—Patiria miniata is a common Pacific Coast starfish with which I have done considerable work. During the spring of 1920 I had not discovered any better method of securing the eggs than that of shaking the excised ovaries in bowls of sea-water. This method had proven rather unsatisfactory because the great majority of eggs thus obtained were immature and incapable of fertilization even after standing for an hour or so. Furthermore, the presence of so many dying and disintegrating eggs tends to foul the water and to encourage the development of deleterious bacterial and fungoid growths, which are far from favorable for the living and active larvæ. This difficulty was formerly obviated to some extent by taking advantage of the fact that there is a certain period in the early larval life when all healthy larvæ swim to the surface and thus may be decanted off and placed in clean sea water. But many of the slightly subnormal larvæ fail to reach the surface and either have to be picked out individually or left to develop amidst the débris of decaying eggs.

The improved method takes advantage of the chance observation that if ripe starfishes are allowed to stand for a few hours out of water they shed both eggs and milt in great volume. The procedure adopted was to place a considerable number of freshly collected starfishes upon a bed of seaweeds in a moist atmosphere. It is found that about ten per cent. of the individuals shed their genital products within from two to four hours and that both eggs and sperms are uniformly ripe and in prime condition, so that essentially all eggs are fertilized and develop normally. Many thousands of eggs may be shed within a few minutes by a single female, making it possible to conduct several experiments with a single batch of eggs. Variety may be secured by fertilizing one lot of eggs with the sperm of several males. It is necessary to use

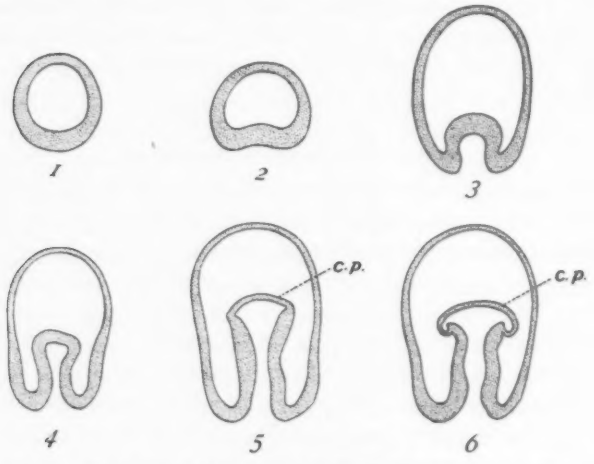
the minimum sperm concentration in order to avoid polyspermy. The standard procedure adopted and used in all experiments was as follows: Five drops of solid sperm, just as it exudes from the genital pores, was mixed with 100 cc. of sea-water; and then ten drops of this sperm suspension was added to a finger-bowl containing a single layer of eggs and 100 cc. of sea-water. Eggs must stand for about an hour in sea-water before insemination is attempted.

Cleavage, Blastula, and Gastrula.

Cleavage begins after about two hours and is entirely similar to that of other asteroids previously described. To get the best results it is necessary to keep the cultures in an unheated room in which the temperature ranges only a degree or so above or below 15° C. Especially is it important that the embryos should not be warmed; a few degrees of lower temperature may be beneficial. The cultures should also be shaded in such a way that no direct sunlight reaches them. When eggs are reared under these precautions they reach a blastula stage in a little under or over 18 hours, and hatch out as swimmers in about 24 hours. The great majority of the blastulæ are practically spherical in form. Even in the best of cultures there are always a few abnormal larvæ that are wrinkled or nearly solid, but in spite of their deformity apparently more active than normal larvæ. It has not been determined whether these abnormal larvæ are the product of overripe eggs, of polyspermy, or of parthenogenesis. It is a simple matter to rid the cultures of all such abnormal larvæ at an early stage, and thus to make conditions better for the normals.

Gastrulation begins a few hours after the larvæ have hatched, and is in no way different from that described for other asteroids. The various steps in the process are shown in Figures 1–6. It is to be especially noted that gastrulæ reared in this fashion are quite free from any apical thickening such as Heath (1918) described for this species and which he considered homologous with the apical plate of the enteropneustan larva. Only subnormal larvæ show this structure, as was pointed out in a previous paper (Newman, 1922), but this becomes especially obvious when improved methods of obtaining eggs are used.

The completed gastrula is somewhat elongated (Fig. 6) and the anterior end of the archenteron reaches about half way to the apical end. This stage is attained at about thirty hours after fertilization.



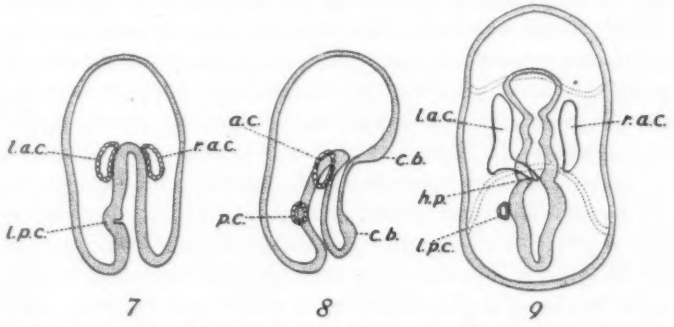
FIGS. 1–6. Early stages, blastulæ and gastrulæ, of *Patiria*. *c.p.*, the thinned-out anterior portion of the archenteron in process of forming the cœlomic pouches.

Post-gastrula Stages.—The changes leading up to the typical Bipennaria condition involve several distinct types of differentiation: (*a*) the development of well-defined dorsal and ventral surfaces, together with a relative shortening of the ventral surface, which thus becomes concave, and a relative lengthening of the dorsal surface, which becomes convex; (*b*) the differentiation of the external ciliated bands; and (*c*) the development of the cœloms.

The larva at about fifty hours (Fig. 8) shows the blastopore moving slightly toward the ventral surface, the somewhat convex contour of the latter, the slight thickening and bulging of the region destined to form the preoral ciliated band, and the enlargement and thinning out of the anterior end of the archenteron to form the pharynx and the anterior cœloms. The changes in larval form and the development of the ciliated bands are so nearly identical with those described for other asteroids that they

may be passed over, at least until more advanced stages are reached. The events associated with the formation of the system of cœlomic pouches, however, need more detailed treatment for they seem to be unique in certain respects.

Development of the Cœlomic Structures.—As has already been said, the first change leading to the formation of cœlomic structures is the enlargement and thinning out of the free end of the archenteron. This is well shown in Fig. 5. The next stage involves a flattening out mushroom-fashion of this thin-walled vesicle, as shown in Fig. 6. Here the paired anterior cœloms are shown in process of being pinched off. This process completes itself, as shown in Fig. 7, by the end of the third day. I have



FIGS. 7–9. Early *Bipennaria* larvæ, showing especially the formation of cœlomic structures. *a.c.*, anterior cœlom; *c.b.*, ciliated band in optical section; *h.p.*, hydropore; *l.a.c.*, left anterior cœlom; *l.p.c.*, left posterior cœlom; *r.a.c.*, right anterior cœlom.

never been able to assure myself, as other writers have done, that the left anterior cœlom is from the first larger than the right. It appears to me that until the formation of the posterior cœlom the larva is perfectly bilaterally symmetrical.

The Posterior Cœlom.—A very significant event, from the standpoint of the development of asymmetry, is the appearance of the posterior cœlomic vesicle. In *Patiria*, this vesicle is a conspicuous object for a considerable period of time and in the end becomes the hypogastric cœlom. This condition is like that described for *Cribrella* and *Solaster* and unlike that in most asteroids thus far described in which the hypogastric cœlom is derived from a

posterior outgrowth of the left anterior cœlom. In *Patiria* the posterior cœlom typically arises on the left side of the archenteron at about the level of the middle of the prospective stomach. It is first seen in the form of a thick-walled evagination of the wall of the archenteron (Fig. 7). This pouch does not expand into a thin-walled vesicle, but merely constricts off a small solid ball of cells that later expands and forms a lumen. The vesicle is always a conspicuous object even under low powers of the microscope, and it affords a ready means of determining the asymmetry of the larva before there are any other positive indications of asymmetry.

Variations in the Position of the Posterior Cœlom.—The typical point of origin of this organ is noticeably to the left of the median line of the archenteron. In many cases, however, it occurs exactly in the median line, under which circumstances it is not infrequently completely double or at least more or less constricted down the mid-dorsal line. Sometimes the double vesicle fuses into one and moves sometimes to the right, but usually to the left, and gives rise to right-handed or left-handed asymmetry. A statistical study of large numbers of larvæ revealed the proportions in which right-handed and left-handed individuals occur. Out of 916 specimens examined, 811 had the posterior cœlom on the left, 98 had it on the right, and 9 had it in a median position or so nearly so that no asymmetry could be made out. This means that over 88 per cent. exhibit left-handed asymmetry, over 10 per cent. right-handed asymmetry, and a little over 1 per cent. show no asymmetry. This is in contrast with conditions described by Gemmill (1915) for *Porania pulvillus*, where "a rudimentary enterocœlic outgrowth arises dorsally by proliferation of the stomach endoderm, usually on both sides of the median line, but sometimes in the median line, or on one side only. . . . When only one body is present it occurs rather oftener on the right than on the left side, a contrast to the condition in *Asterias rubens*, where the corresponding body (a still smaller one) appears most commonly on the left side." It seems evident from these observations that the presence among the asteroids of the posterior cœlomic vesicle and its asymmetry exhibits a highly variable incidence. The body is one that seems to be relatively vestigial in the group as a whole, but appears in a *Cribrella*,

Solaster, and *Patiria* (doubtless also in other species) to be rather large and of some functional importance. It should also be noted that (in *Patiria*) the asymmetry exhibited by this vesicle always coincides with that exhibited by other cœlomic structures.

Development of Hydropores and Pore-canals.—On about the fifth day the larva gives off, typically from the posterior end of the left anterior cœlom, a dorsally directed hollow process, which comes into contact with the larval body wall and breaks through to form an open hydropore (Fig. 9). In *Patiria* the pore canal and hydropore constitute a large and conspicuous landmark for the determination of asymmetry. Without a single observed exception the hydropore is formed on the same side as the already formed posterior cœlom. There were a few cases in which the posterior cœlom appeared to be median, while the hydropore appeared only on one side. Attention should be called here to the fact that, while in *Poranus* (Gemmill, '15) the hydropore appears a day or so earlier than the posterior cœlom, exactly the opposite time relation prevails in *Patiria*, the posterior cœlom appearing a day or so earlier than the hydropore. Probably the earlier appearance of the posterior cœlom in *Patiria* accounts for its larger size and its permanency.

As was the case with the posterior cœlom, the hydropore also shows reversed asymmetry. The reversed or right-handed larvæ are as large, as vigorous, and as healthy as are the more typical individuals. A census of 652 individuals, belonging to seven different lots of larvæ, showed 589 with left-hand hydropores, 63 with right-hand hydropores, and none with both right-hand and left-hand hydropores; which means that over 90 per cent. are left-handed, and less than 10 per cent. are right-handed. We see then that the percentage of individuals with completed right hydropore is somewhat less than that of individuals with right posterior cœlom, but the difference is not significant, as both are very close to 10 per cent. Furthermore it may be said that the above censuses were made upon different lots of larvæ from those given for controls in the experimental part of the paper, and strongly tend to confirm the latter.

It should strongly be emphasized in this connection that the right-hand hydropores in *Patiria* are not merely temporary

structures that subsequently regress and give place to left-hand hydropores, as Gemmill claims to be the case for *Asterias rubens*, but are permanent asymmetrical structures that remain up to the point of metamorphosis. In every culture observed the right-handed individuals maintained their reversed asymmetry and developed on the average as normally as did the more typical left-handed larvæ; in fact one gets the impression that in some cases at least, the reversed larvæ are a little larger and more active than the typical ones belonging to the same lot.

This is, I believe, the first time that large numbers of individuals with right-handed (reversed) asymmetry have been described for echinoderms. Only very rare and sporadic instances of reversal of asymmetry have been reported. In *Poranus*, a species of starfish studied intensively by Gemmill, double hydropores occurred in about 50 per cent. of the larvæ, but he specifically states that but "a single instance was observed in which the hydropore occurred on the right side alone." It may be pertinent, however, to recall that Oshima found both in control laboratory cultures and in experimental cultures of *Echinus miliaris* about 10 per cent. of specimens with right-hand asymmetry. In these experiments, however, it must not be forgotten that both controls and experiments were conducted under distinctly abnormal conditions, since artificial sea-water was used and the food was unsatisfactory. The possibility is not to be denied, however, that this species of echinoid may exhibit normally a fairly high degree of reversed asymmetry.

From what has been said, it may be inferred that *Patiria miniata* is in a somewhat delicate state of organic equilibrium with regard to its asymmetry, and should, therefore, furnish ideal material for an experimental analysis of the nature of this asymmetry.

The Development of the Hydrocœle and its Rays.—Although one may observe by the end of the first week or ten days a posterior prolongation of the left (in reversed larvæ, the right) anterior cœlom, this structure does not thicken up and become obviously a hydrocœle until about the end of the third week. In fact, a hydrocœle never develops unless the larvæ are well fed and otherwise well cared for. The hydrocœle appears at the same

time and in the same manner in right-handed as in left-handed larvæ.

The Form of Bipennaria and of Brachiolaria Larvæ.—The form of the *Bipennaria* on the fifth day is shown in Fig. 9. It may be noted that the blastopore or larval anus has moved around to a distinctly ventral position; that the hydropore and pore-canal are well defined; that the posterior cœlom is now distinctly vesicular; and that both preoral and postoral ciliated bands have begun to be differentiated.

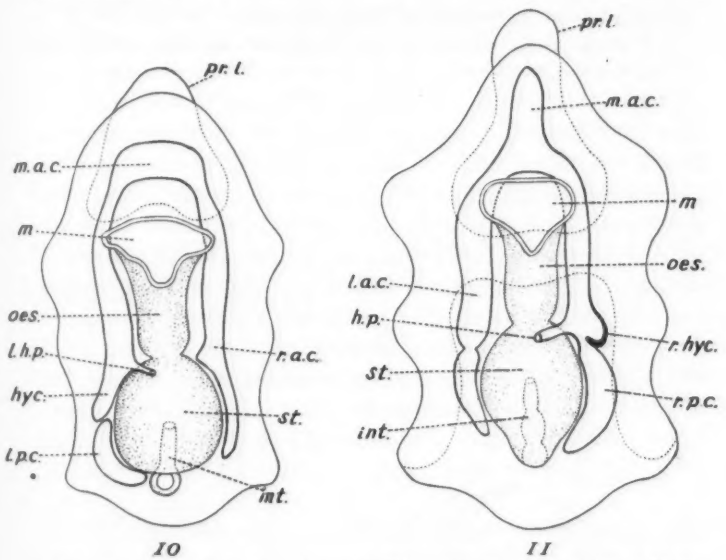


FIG. 10. Dorsal view of an advanced *Bipennaria* showing the ordinary left-handed asymmetry.

FIG. 11. A somewhat more advanced *Bipennaria* seen from the dorsal side and showing reversed or right-handed asymmetry. *h.p.*, hydropore; *hyc.*, hydrocœle; *int.*, intestine; *l.a.c.*, left anterior coelom; *l.h.p.*, left hydropore; *l.p.c.*, left posterior cœlom; *m*, mouth; *m.a.c.*, median anterior cœlom; *oes.*, œsophagus; *pr.l.*, preoral lobe; *r.a.c.*, right anterior cœlom; *r.hyc.*, right hydrocœle; *r.p.c.*, right posterior cœlom; *st.*, stomach.

At nine days the larva has undergone much change. The right and left anterior cœloms have grown forward beyond the mouth. The left (in reversed larvæ, the right) anterior cœlom has extended backward so as to be in contact with the posterior

cœlom; while the right anterior cœlom has a posterior prolongation extending as far as the posterior end of the stomach. The preoral ciliated band is triangular and the posterior end of the postoral band is turned well forward toward the mouth.

At two weeks (Fig. 10) the anterior cœloms of the two sides have fused above the pharynx to form a continuous vesicle. The left anterior cœlom is still separate from the posterior cœlom, which is now quite large. The posterior prolongation of the right anterior cœlom has become relatively small in caliber, in fact has begun the process of rudimentation.

At twenty-four days (Fig. 11) the left anterior cœlom has

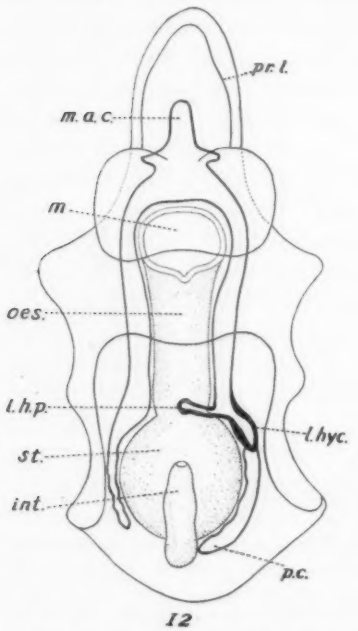


FIG. 12. Advanced *Bipennaria* larva seen from the ventral surface, showing left hydrocœle well developed.

FIG. 13. Young *Brachiolaria* larva, seen from the dorsal surface, with reversed asymmetry. Primitive tentacles (*p.t.*) are shown arising from the hydrocœle of the right side. Right hydropore (*r.h.p.*) and right posterior cœlom (*r.p.c.*) The preoral lobe has become modified into the attachment organ of the simplified *Brachiolaria*, with median sucker (*m.s.*) and lateral suckers (*l.s.*). The remaining lettered structures are explained in previous legend.

united with the posterior cœlom; the hydrocœle has begun to undergo marked thickening of its walls; the median anterior part of the fused anterior cœloms has sent forward a long median process into the preoral lobe of the larva which has now a trilobed shape.

At twenty-six days (Fig. 12) the median diverticulum of the anterior cœloms has branched terminally into three parts, a large median one and two small lateral ones. The preoral lobe is now decidedly a trilobed affair.

At the end of a month the larva takes on the characteristic features of a simplified *Brachiolaria* (Fig. 13). The changes that signal the arrival of the larva at the Brachiolaria stage have to do with the differentiation of the median and the two lateral brachia of the preoral lobe, organs that were already foreshadowed in the trilobed form of the preoral lobe and in the three-branched condition of the median anterior cœlomic process. The middle brachium develops a well-defined attachment disk (Fig. 16) on which are present three pairs of papillæ. The lateral brachia remain simple knobs, slightly thickened and clubbed at the ends. One branch of the anterior extension of the cœlom runs into each brachium, but the lateral branches remain relatively small. A ventral sucker appears between the paired lateral brachia. The hydrocœle of the *Brachiolaria* has become lobed in some individuals, and in others it has given off some or all of the radial branches, destined to form the radial water canals (Figs. 13, 14, 15).

Further than this it was not possible to go with certainty. For one month the larvæ grew normally on a diet of the diatom, *Nitschia*, but almost over night the best cultures underwent retrogressive changes and died. One individual was found which had rounded up into a thin-walled vesicle. At first it was hoped that this individual had undergone or was undergoing metamorphosis, but in less than a day it also died. The same fate attended three other carefully reared lots of larvæ, all dying after about a month, and none, so far as I was able to determine with certainty, completing the process of metamorphosis. In spite of this ill fortune it should be emphasized that larvæ with right-handed (reversed) asymmetry were found among the most

advanced of the *Brachiolariæ*, and would without doubt have developed the mouth and other oral structures of the adult from the right-hand side of the larva. If this type of symmetry

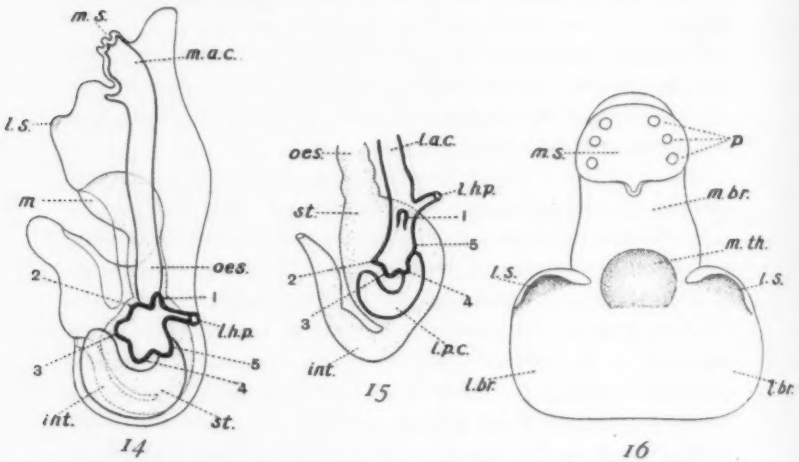


FIG. 14. *Brachiolaria* larva seen from the left side. The primitive tentacles of the prospective adult are numbered 1, 2, 3, 4, 5. Other lettered structures the same as in previous legends.

FIG. 15. Posterior portion of another *Brachiolaria* larva, showing another stage in the formation of the prospective adult.

FIG. 16. Ventral external view of the preoral lobe of *Brachiolaria* shown in Fig. 14. The median brachium (*m.br.*) has a median sucker (*m.s.*) with six papillæ (*p*). There are two short lateral brachia (*l.b.*) each with a poorly developed lateral sucker (*l.s.*). Between the lateral suckers is a median thickening (*m.th.*) which may be another sucker.

reversal takes place sometimes in nature, as it doubtless does, there appears to be nothing about the anatomy of the radially symmetrical adult to indicate whether it has been derived from a larva with the normal or one with the reversed asymmetry.

In attempting to account for repeated failures to rear these asteroid larvæ through the period of metamorphosis, it has occurred to me that in nature there doubtless takes place a radical change in the dietary of the young individuals, which up to metamorphosis had been herbivorous, feeding largely upon diatoms, and that after metamorphosis they change to a carnivorous diet; for the adult is strictly carnivorous. Hence

failure to metamorphose may be due to the absence of the forms of animal food essential for this process.

III. Experimental Induction of Reversed Asymmetry and of Bilaterality in *Patiria*.

Materials and Methods.

In all experiments dealt with in this paper the eggs of one female fertilized by the sperm of one male were used both for experimental and control lots. Large numbers of fully ripe eggs and sperm are readily obtained in the manner described in the introduction to this paper.

After several preliminary trials it was found that the blastula stage is the one best adapted for the experimental induction of reversals of asymmetry. Consequently all experiments herewith presented were performed upon early, middle, and later blastulæ, and early gastrulæ.

The experimental procedure was as follows: An ice box containing a large, flat layer of ice was the only unusual apparatus employed. A single lot of eggs was divided into two, three, or four equal lots, care being taken to avoid crowding. As a rule, a single layer of eggs on the bottom of the dish was found to be not too many to permit of normal development. One lot was always kept as a control, being placed from the time of fertilization on in an unheated room, resting upon a concrete table and shaded from too strong light. The experimental lots contained in covered glass vessels were placed directly upon the block of ice. After about half an hour temperature readings were taken and it was found that the water at the bottom of the dish where the embryos lay ranged from 2° to 3° C., this being about the temperature of the surface of the ice itself. At this temperature all progressive development comes to a complete standstill, but is resumed as soon as the normal growth temperatures are reinstated. In spite of the fact, which will be more fully dealt with in a subsequent paper, that development is inhibited at the experimental temperatures, the ciliated larvæ lived and remained active in some cases for as long as ten days, though kept continuously on ice for the entire period. This circumstance is mentioned here merely to

emphasize the point that, though development ceases, certain phases of vital activity continue almost unabated.

In presenting the experimental results it seems advisable to give in some detail the results of the first complete experiment, which afterwards proved to be typical, and to follow this with a relatively condensed account of several nearly identical experiments that serve to corroborate the findings of the more intensively studied first experiment.

Experiment 1.

A fine batch of eggs, fertilized at 2.15 P.M., April 6, 1923, was divided into four equal lots. After 17 hours, when the embryos were still unhatched blastulæ, but were rolling about within the vitelline membrane, three lots were put on ice and one lot kept under normal conditions as a control. One lot (1, *A*) was kept on ice for one hour, a second lot (1, *B*) was iced for two hours, and a third lot (1, *C*) was iced for three hours. After exposure to low temperatures the experimental lots were placed beside the control lot in a room with temperature of about 15° C. and allowed to develop without disturbance for five days, at which time the first definite signs of asymmetry may be observed. On the fifth day both the control and the experimental lots were carefully looked over with reference to the percentage of larvæ showing reversal of asymmetry. It was obvious under casual observation that there were many more reversed larvæ in the experimental lots than in the control, but the exact difference could be determined only by means of a census of a representative number of individuals from each lot.

The plan adopted was that of random sampling. A few larvæ at a time were picked up in a pipette and transferred to a Syracuse watch glass and all examined to determine the position of the posterior cœlom, a rather conspicuous object at this time. When the count reached 100 the census was considered complete. This might be considered too small a number to give significant data, but when this is repeated a great many times it gains statistical value.

First Census (5 days old).

Control	87%	of larvæ	have	left	posterior	cœlom	
	13%	"	"	right	"	"	
1, *A* (iced 1 hr.)	56%	"	"	left	"	"	
	44%	"	"	right	"	"	
1, *B* (iced 2 hrs.)	79%	"	"	left	"	"	
	21%	"	"	right	"	"	
1, *C* (iced 3 hrs.)	36%	"	"	left	"	"	
	14%	"	"	right	"	"	
	50%	"	"	median	"	"	

Note: In the last lot it was impossible to decide in just half of the larvæ whether the posterior cœlom was destined to go to the left or to the right. The culture was somewhat belated and at this time and the asymmetry matter was unsettled in many of the larvae. Yet those that have settled their asymmetry show 72 per cent. with left-hand asymmetry as compared with 28 per cent. with right-handed asymmetry, a result very closely consistent with that shown in the second census of the same lot.

Second Census (six days old).

Control	97%	of larvæ	have	left	water	pore	and	pore	canal
	3%	"	"	right	"	"	"	"	"
1, *A* (iced 1 hr.)	71%	"	"	left	"	"	"	"	"
	29%	"	"	right	"	"	"	"	"
1, *B* (iced 2 hrs.)	74%	"	"	left	"	"	"	"	"
	26%	"	"	right	"	"	"	"	"
1, *C* (iced 3 hrs.)	72%	"	"	left	"	"	"	"	"
	13%	"	"	right	"	"	"	"	"
	15%	"	"	both right and left water pores and pore canals					

Third Census (ten days old).

Control	98%	of larvæ	with	left	asymmetry
	2%	"	"	right	"
1, *A* (iced 1 hr.)	89%	"	"	left	"
	11%	"	"	right	"
1, *B* (iced 2 hrs.)	88%	"	"	left	"
	12%	"	"	right	"
1, *C* (iced 3 hrs.)	61%	"	"	left	"
	21%	"	"	right	"
	18%	"	"	bilateral symmetry	

Note 1.—At this time it was evident that the experimental larvae, especially those in Lot 1, *C*, were smaller and less advanced than the control. It was necessary to discard from those chosen for the census all distinctly abnormal larvæ and those in which no water pores or pore canals had developed, and there were many of these. The suppression of these structures in experimental lots is in itself an important result, and is considered simply a more marked expression of the same morphological response that is shown in disturbances of symmetry.

Note 2.—In connection with the 18 per cent. of bilateral larvae listed for Lot 1, *C* in the last census, it should be noted that the paired water pores and pore canals were rarely equal in size, sometimes that of the left side and sometimes that of the right side being larger.

Summary of Experiment 1.

1. There was a fairly high percentage of control larvæ in the first census showing reversed asymmetry of the posterior cœlom, but the numbers of control larvæ with reversed asymmetry in the later censuses was very small, only 3 per cent. and 2 per cent. respectively.

2. In every census (9 in all) the experimental cultures showed a much higher percentage of reversed larvæ than the controls. The average per cent. of reversed larvæ in experimental lots was 30.44, as compared with 4.66 for the controls. Thus there was over six times as high a percentage of symmetry reversal in experimental lots as in controls.

3. In Lot 1, *C* (iced 3 hours) there were many bilateral larvæ, only one of these appearing in any of the other lots.

4. The conclusion seems obvious that in this experiment the percentage of instances of symmetry reversal has been very markedly increased by retarding the development of the blastulæ. In other words, reversal of asymmetry and bilateral symmetry have both been induced experimentally.

5. To determine whether this experiment is exceptional or typical, eight other experiments were carried out using the same materials and methods, but with some variations in the stages at which the larvæ were iced and in the length of exposure to low temperatures.

Experiment 2.

Twenty-one hours after fertilization, when all larvæ were hatched and swimming about as late blastulæ and early gastrulæ, they were divided into three lots. One was left as a control, a second iced for one hour, and a third iced for two and one half hours.

First Census (5 days old).

Control	94%	of larvæ	with	left	posterior	cœlom
	6%	"	"	"	right	"	"
2, *A* (iced 1 hr.)	81%	"	"	"	left	"	"
	6%	"	"	"	right	"	"
	13%	"	"	"	median	"	"
2, *B* (iced 2.5 hrs.)	63%	"	"	"	left	"	"
	31%	"	"	"	right	"	"
	6%	"	"	"	median	"	"

Second Census (One week old).

Control	92%	of larvæ	with	left	water	pore	and	pore	canal	
	8%	"	"	right	"	"	"	"	"	
2, *A* (iced 1 hr.)	86%	"	"	left	"	"	"	"	"	
	13%	"	"	right	"	"	"	"	"	
	1%	"	"	bilateral	"	"	"	"	"	
2, *B* (iced 2.5 hrs.)	76%	"	"	left	"	"	"	"	"	
	24%	"	"	right	"	"	"	"	"	

Experiment 3.

Twenty hours after fertilization, when all larvæ were free-swimming blastulæ or early gastrulæ, they were divided into three lots: one the control, one iced one and a quarter hours, and the third iced two and a quarter hours.

First Census (5 days old).

Control	84%	of larvæ	with	left	posterior	cœlom	
	16%	"	"	right	"	"	
3, *A* (iced 1.25 hrs.)	69%	"	"	left	"	"	
	24%	"	"	right	"	"	
	7%	"	"	median	"	"	
3, *B* (iced 2.25 hrs.)	77%	"	"	left	"	"	
	19%	"	"	right	"	"	
	4%	"	"	median	"	"	

Note: There was one specimen in Lot 3, *B* (iced 2.25 hrs.), which had two full-sized posterior cœloms on the left side, one behind the other. This might be interpreted as incipient metamerism.

Second Census (one week old).

Control	87%	of larvæ	with	left	asymmetry	
	13%	"	"	right	"	
3, *A* (iced 1.25 hrs.)	81%	"	"	left	"	
	16%	"	"	right	"	
	3%	"	"	bilateral	symmetry	
3, *B* (iced 2.25 hrs.)	83%	"	"	left	asymmetry	
	14%	"	"	right	"	
	3%	"	"	bilateral	symmetry	

Note: This experiment shows only a slight increase in the percentage of reversed asymmetry through icing. It should be noted, however, that a large percentage of the larvæ, though not quite so old as those in Experiment 2, were beginning gastrulation and thus past the period of maximum susceptibility to symmetry reversal. This will be shown more clearly in Experiments 8 and 9.

Experiments 4, 5, 6, 7, 8, 9.

Six different lots of eggs from six different females, fertilized by the sperm of one male, were dealt with exactly alike except that some were allowed to develop a few hours longer before icing, and

varying times of exposure to low temperatures were tried. Each culture was divided evenly into two lots, one for control and one for experiment. As all lots were allowed to develop for a week or more before the census, the results may be readily tabulated as though belonging to one census though it took about three days to complete the count.

TABLE I.

No. of Exper.	Stage at which Iced.	Time Iced.	Symmetry of Larvæ when Examined.
4.....	Early blastulæ	2 hrs.	Control—89% left asymmetry 11% right " Iced—76% left " 24% right "
5.....	Early blastulæ	3 hrs.	Control—83% left " 17% right " Iced—73% left " 27% right "
6.....	Advanced blastulæ	7 hrs.	Control—87% left " 13% right " Iced—72% left " 24% right " 4% bilateral symmetry
7.....	Advanced blastulæ	18 hrs.	Control—93% left asymmetry 7% right " Iced—69% left " 21% right " 10% bilateral symmetry
8.....	Early gastrulæ	1 hr.	Control—88% left asymmetry 12% right " Iced—87% left " 13% right "
9.....	Later gastrulæ	6 hrs.	Control—91% left " 9% right " Iced—93% left " 7% right "

SUMMARY OF TABLE I.

(*a*) In Experiments 4, 5, 6, and 7, in which blastulæ were used, the iced lots showed a marked increase of symmetry reversal over the controls. Including those showing bilaterality among the reversed types, there are over 27 per cent, on the average, of reversed larvæ, as compared with 12 per cent. for the controls.

(*b*) In Experiments 8 and 9, where gastrulæ were used, there was no significant increase or decrease in the percentage of larvæ with reversed asymmetry as the result of low temperatures. This is in accord with Experiment 3, in which a large proportion of the larvæ were gastrulæ and in which the increase in reversed larvæ was relatively slight.

(*c*) Only in Experiments 6 and 7, where the larvæ were exposed for long periods to low temperatures (7 and 18 hours, respectively) were any larvæ found with water pores and pore canals on both sides. It might then be said that bilaterality is harder to induce than reversal of asymmetry.

IV. Discussion of Experimental Results.

Patiria miniata is a species of starfish that either exhibits in nature a fairly high degree of reversed asymmetry or else is so susceptible to influences tending to affect the symmetry system that even control cultures cannot be reared without the induction of some degree of symmetry reversal. Three experiments were tried with the idea of determining to what extent symmetry reversal could be reduced by taking every conceivable precaution against conditions that might be subnormal. Using only the freshest eggs and a minimum sperm concentration, allowing only a few eggs to a dish, keeping the dishes in running sea-water, feeding the larvæ on a pure culture of the diatom, *Nitschia*, and introducing some fresh sea-water daily, there was found to be on the average about 8 per cent. of larvæ showing reversed asymmetry, which is not appreciably smaller than that seen in the controls in the various lots previously dealt with. One may conclude then that *Patiria* is a species in which left-handed asymmetry is not fully fixed, but that the symmetry system is in a somewhat plastic state, a state well adapted for experimental purposes.

Left-handed asymmetry is at the present time normal for echinoderms. Considerable work has been done which tends to prove that this asymmetry is present even in the unsegmented egg, and also that the first cleavage divides the egg into the prospective right- and left-hand sides. When twins are produced by the isolation of the blastomeres at the two-cell stage it has been found that one twin develops faster and becomes a more advanced larva at any given time than does the other. It seems then that one half of the egg, one of the two blastomeres, and one half of the larva is physiologically superior to the other in the sense that it has a higher general metabolic rate as expressed in more rapid growth. As a rule the superior side is the left side.

Studies of the development of double monsters, which are incompletely divided twins, have shown that, in these bilateral systems, whenever one component for any reason gains a physiological ascendancy over the other, there is a strong tendency for the superior component to remain normal and for the inferior individual to become subnormal and to be reduced to a condition

of a mere parasite upon the normal component. Thus arises the condition known as that of the "autosite and parasite" type of double monsters. In extreme cases of this sort the "parasite" component is reduced to a rudimentary cyst embedded in the tissues of the parasite. This condition I have already interpreted as one of *dominance and subordination* in growth. According to this principle, which has been shown to hold good in large numbers of cases in both animals and plants, a rapidly growing or differentiating region, or apical region, in an organism tends to inhibit the growth or differentiation of like regions within the realm of its influence. This is true of equivalent bilateral regions, which may be conceived of as intense rivals. If one rival region gains an ascendancy over the other, the inferior region tends to be suppressed.

Applying this principle to echinoderm development, we can readily recognize the analogy to the "autosite and parasite" situation in double monsters. Commonly the left side acts as the superior or "autosite" component, and the right side as the inferior or "parasite" component. The result is that certain important and actively differentiating structures (the posterior cœlom, the hydropore, and the hydrocœle) appear first on the more rapidly developing left side, and that these structures suppress the development of equivalent structures on the right side and tend to reduce to vestiges certain other cœlomic structures of the right side that have already appeared. In other words, the typical adult echinoderm is morphologically the surviving left-hand component of a twin or double monster, and its radial symmetry is merely a sort of mechanical adjustment necessary in order to avoid too pronounced a one-sidedness.

If, however, the rate of growth of a larva be sharply checked in such a way that the physiological state of the right and left components are reduced to a parity, it should be possible to get an organism both halves of which are equivalent. As a matter of fact, this result is not infrequently realized in the bilateral larvæ that have arisen as the result of rather prolonged icing. The commoner result, however, is that the right side usurps the ascendancy typically belonging to the left side and gives rise to larvæ with reversed asymmetry. If the physiological condition

of the two sides were really reduced to parity, we should always have, as the result of icing, bilateral larvæ; but we should not, a priori, expect a retarding agent to effect the two sides equally. On the contrary, the expectation would be that the superior side would be more seriously affected than the inferior, so that when recovery took place the originally inferior side would emerge from the growth retarding influence first and would then behave like a physiologically superior side throughout development. Two difficulties immediately present themselves. Why, it may be asked, are not all larvæ reversed? And why do not the right-handed larvæ, about ten per cent. of which seem to be present in each lot, reverse and become left-handed?

The second of these queries may best be answered first by admitting that there is no ground for denying that at least some of the right-handed individuals, under the conditions of the experiment, do reverse their already reversed asymmetry back to the specific left-handed asymmetry. If this be taken into account, we would have to add materially to the observed percentages of reversed larvæ, for a certain percentage of the left-handed larvæ that are counted as unreversed are doubtless reversed right-handers. But there is still a large percentage of larvæ that have failed to undergo symmetry reversal, and our problem is to account for their failure to reverse. The only solution of the problem is one that takes into consideration the well-known fact that no two eggs or embryos are alike in the degree of their susceptibility to inhibiting agents or in their responses to given agents. It is probable that only a limited number of larvæ at any one time are in just that state of susceptibility the normal response to which is symmetry reversal, and that these unsusceptible types are responsible for the surplus of left-handed larvæ in all experimental cultures. Other larvæ show other expressions of growth inhibition, while still other particularly hardy forms show no effects at all.

The problem that confronts us in this connection is in no essential respect different from the one that has faced experimental embryologists ever since the first eggs or embryos were subjected to conditions other than normal. It makes no difference whether one uses toxic chemicals, anæsthetics, low

temperatures, or hybridization, the result is always the same: some individuals are affected in one way, some in another, and some not all. A lot of Fundulus eggs, for example, are exposed for a given time to a given intensity of X-rays. Some of the radiated individuals, as Miss Hinrichs has shown, develop into two-headed monsters, others become cyclopic, others show lesions of the heart or circulation, not to mention many other typical teratological conditions. Our present problem does not concern itself with any attempt to account for the individual differences in susceptibility among eggs of the same batch: this is a problem of heredity, not one of development.

To revert specifically to the present experiments, it should now be said that, although our attention has been focused upon symmetry reversal to the exclusion of any other effects of exposure to low temperatures, it would be unfortunate to leave the reader with the impression that everything else about the experimental larvæ was strictly normal. The truth is that some larvæ develop no cœlomic structures at all; that others show a tendency toward a metameric duplication of anterior or posterior cœloms; that occasionally a larva has a hydropore connected with the posterior cœlom as well as the anterior. It should also be added that in all experiments in which the icing of larvæ lasted more than about two hours, many other expressions of growth inhibition appeared, sometimes associated with symmetry reversal and sometimes not. My strong impression was that on the whole the larvæ with reversed asymmetry were rather more normal in other respects than those with typical asymmetry.

In conclusion then, it may be said that symmetry reversal, and that particular phase of it which we may call induced bilaterality, are merely some of the specific effects resulting from arresting the growth of the larvæ at the blastula stage. If the arrest takes place much earlier or much later than the blastula stage other expressions of differential inhibition prevail and there is no evidence of symmetry reversal. If eggs are iced during early cleavage or even before cleavage the particular response is *twinning*, while icing at other times give equally striking but different results. These matters, however, must be left for another study and another report.

One of the principal values accruing from experimental embryology is that it often throws light upon the mechanism of normal development. It would be strange, then, were we to fail to find in this study no suggestion as to a more complete understanding of the significance of asymmetry in the echinoderms and of that remarkable series of changes involved in metamorphosis. I venture, therefore, to conclude this paper with an attempt to reinterpret in physiological terms the phenomenon of echinoderm metamorphosis in general.

V. A Physiological Interpretation of Echinoderm Metamorphosis.

There are many remarkable ontogenetic transformations in nature, but none so radical as the metamorphosis of the echinoderm larva into the adult. In the metamorphosis of the caterpillar into the butterfly, the axes of polarity and of symmetry are carried over unchanged from larva to adult; in the metamorphosis of the tunicate tadpole larva into the adult sea-squirt there is a relative inhibition of the apical regions of both axes, yet the polarity and symmetry of the larva are maintained in the adult; but in the metamorphosis of the echinoderm both polarity and symmetry of the larva are, as it were, entirely ignored, and the axes of the adult are established practically *de novo* and without much reference to previously existing axiate relations.

In terms of the physiological gradients involved, it may be said that what apparently happens is, that the original gradients of the egg and of the larva become practically obliterated and a new major axis arises approximately at right angles to the larval major axis with its apical region at a point somewhere near the middle of the left side of the larval stomach at the point where the hydrocœle arises. The crucial event in this shifting of axiate relations is, morphologically speaking, the differentiation of the hydrocœle. This structure, as all know, arises as an outpouching of the posterior end of the left anterior cœlom (enterocœle). There comes a time in late larval life when all other growth changes cease and the enlargement and differentiation of the hydrocœle seem to be the main changes taking place. This rapidly growing region then assumes dominance over the rest of

the organism, becomes the apical point of a new axis, and assumes control of the entire subsequent development. For some time after the new or adult axis has arisen the original larval axes are maintained in so far as the general external form and behavior of the individual are concerned, but sooner or later these yield to the increasing dominance of the new apical region, as may be seen by the casting off or resorption of the most characteristic features of the larva, such as the ciliated arms or bands, the preoral lobe with its mouth and accompanying structures, and in the rudimentation of the cœlomic structures of the right side.

The developmental history of the hydrocœle may briefly be reviewed. Physiologically, it represents a region of rapid cell proliferation in a larva which is, at the time in question, practically at a developmental standstill. As is well known, any rapidly growing region in an organism has the capacity of dominating the development of other regions about it and of suppressing the differentiation of similar or homologous regions elsewhere in the organism. It may also, in the course of time, inhibit the development of rival regions and cause their atrophy or resorption. Morphologically speaking, the events are as follows: The hydrocœle, at first a small lobe of the left anterior enterocœle, buds off five primary tentacles—destined to become the five radial water canals. These canals assume a radiate position about a central ring through which the new mouth passes. It is about this ring and its five—more in some species—primary tentacles that the new adult radial organization centers. The primary axis of the adult passes through the center of the water-vascular ring. This new axis can be called neither an antero-posterior axis nor an axis of symmetry; it is something quite *sui generis*, as is recognized by those who have given to the two poles the names "oral" and "aboral." The secondary axis is also quite unique, for there is no true symmetry, but merely a superficial semblance of radial symmetry.

Symmetry Relations of Larva and Adult.—From the standpoint of the original larval axes of symmetry, it is clear that the events that lead up to metamorphosis occur typically only on the left side. On this account the situation has long been thought of as an outstanding example of asymmetrical development, and there

has been much speculation as to its morphological and its phylogenetic significance. Prominent among the speculations that have arisen in connection with this phenomenon are those that concern themselves with the phylogeny of the echinoderms. It is generally held that the ancestral echinoderm was, like the larva, bilaterally symmetrical and that hydrocœles and their derivatives occurred on both sides. The objection to this view is that such an organism would be biaxiate, with two mouths and oral surfaces, two water-vascular systems and nerve rings. In view of this serious objection, it would seem best to look upon the development of the new axis as an evolutionary process resulting from the establishment of a pronounced asymmetry, and that it was only in connection with this newly established asymmetry that the development of such structures as the new mouth, the nerve ring, the Aristotle's lantern in echinoids, and similar structures, takes place. This view does not preclude the possibility of an originally bilateral ancestor, but merely denies to such an ancestor the bilateral possession of such specialized structures as the hydrocœle and its derivatives that arise only in connection with the apical region of an entirely new axis.

When, however, larvæ are found with hydrocœles and kindred structures on both sides, evidence *seems* to be afforded for the theory that the ancestral echinoderm had two sets of hydrocœle structures bilaterally arranged. If such anomalous larvæ are to be considered as atavistic, how can we explain the occurrence of larvæ in which the hydrocœle and its appurtenances appear exclusively on the right side? Is the right-handed or reversed larva also an atavistic reminiscence of an ancestor that had right-handed asymmetry? Even the most obdurate phylogenist would hesitate to go so far; yet the occurrence of reversed asymmetry is commoner than is bilaterality, and both can be induced by the same experimental means. Moreover, the two conditions grade into each other; for there are many larvæ in which hydropores and such structures occur on both sides but in which one or the other side shows these structures in all states of partial rudimentation.

How then can we explain the occurrence both in nature and in experiments of reversed and bilateral larvæ? The previous parts

of this paper have indicated that all anomalies of this kind are merely induced developmental disturbances and that neither bilateral nor reversed larvæ have any more phylogenetic significance than has the occurrence of occasional two-headed fishes or than the sporadic occurrence of sinistral asymmetry in a species of snail typically dextral.

The Nature and Origin of Asymmetrical Development.—The question naturally arises as to why one side of an echinoderm larva should develop structures not duplicated on the other. Two possible explanations suggest themselves: first, that the cells constituting one half of the larval body have a higher rate of metabolism and hence a somewhat more rapid rate of proliferation and of differentiation than those of the slower side; second, that the asymmetry is due to some environmental factor that influences one side only or the two sides differently. In support of the first explanation it should be said that the cells destined to form the two bilateral halves of the larva are set apart from a very early period. Driesch (1906) found that bilaterality was definitely established at the eight-cell stage, and presented data that tended to show that the first cleavage plane coincides with the sagittal plane of the larva. In a number of cases he found that when twin gastrulæ were formed from the two blastomeres these twins showed mirror-image symmetry. This observation I have been able to confirm in connection with studies of twinning in *Patiria* (Newman, '22). Bilateral symmetry then appears to be established at the beginning of cleavage and is probably predetermined in the unsegmented egg.

More significant still for our problem is the fact that, in a large proportion of twins produced from the physiologically isolated blastomeres of the two-cell stage, the rate of development of the two larvæ is markedly different. Almost invariably one of a pair of such twin blastulæ or gastrulæ within a common vitelline membrane is well in advance of the other. In an earlier stage it can be seen that one twin blastula has fewer cells and a smaller blastocœle than the other. In later stages one gastrula is relatively normal and quite active, while the other is not infrequently subnormal and shows signs of disintegration.

These data indicate that the asymmetry which is in later stages

expressed in the unilateral appearance of the hydrocœle and its derivatives, is traceable to a very early stage of development and is probably present in the unsegmented egg. Moreover, it appears merely to consist of an inequality of metabolic rate between the antimeric halves of egg and embryo. The difference between the two sides is in no sense qualitative, as some writers prefer to believe, but is purely quantitative; for, as we have seen, all the structures that characterize the superior side may be inhibited on that side and may be thus allowed to appear on the other side, or they may appear on both sides at once in case the physiological state of the two sides is experimentally equalized. The basis of asymmetry in the typical echinoderm is then the result of a dynamic imbalance between the earliest primordia of the prospective antimeric halves of the embryo. On this account one side develops more rapidly than the other, especially in the region where the hydrocœle arises, and the differentiation of the hydrocœle and its derivatives tends to suppress the growth of equivalent structures on the opposite side. This view is consistent with the experimental work reported in this paper and with a large mass of morphogenetic work of other sorts. No claim of finality is made for the conclusions reached in this study, but it is hoped that some of the ideas expressed may form a starting point for future investigation.

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